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MILK QUALITY AND MASTITIS IN JIMMA, ETHIOPIA- RISK FACTORS AND CONSTRAINTS

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There are no secrets to success. It is the result of preparation, hard work, and learning from failure. (**Colin Powell**)

DEDICATED

To my father and mother:

Tolosa Fulasa and Alemi Tolosa,

who never went to school themselves for raising me up and sending me to school.

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List of abbreviations

^{0}C	Degree Celsius
BCS	Body condition score
BTSCC	Bulk milk tank somatic cell count
BTTBC	Bulk milk tank total bacterial count
CAMP	Christie, Atkins, Munch-Petersen
CC	Coliform count
CFU	Colony forming unit
CI	Confidence interval
CMT	California mastitis test
CNS	Coagulase-negative *Staphylococcus*
CPS	Coagulase-positive *Staphylococcus*
CSA	Central Statistical Agency
EU	European Union
FAO	Food and Agriculture Organization
IMI	Intramammary infection
IQR	Interquartile range
IUC-JU	Institutional University Cooperation -Jimma University project
LPC	Laboratory Pasteurized Count
MRSA	Methicillin Resistant *Staphylococcus aureus*
NMC	National Mastitis Council
OR	Odds ratio
ORSAB	Oxacillin resistance screening agar base
PCR	Polymerase chain reaction
PIC	Preliminary Incubation Count
QSAE	Quality and standard authority of Ethiopia
Ref	Reference
SCC	Somatic cell count
SCM	Subclinical mastitis
SPC	Standard plate count
TBC	Total bacterial count
VLIR-UOS	Vlaamse Interuniversitaire Raad-University Development Cooperation
WHO	World Health Organization

Chapter 1:

General introduction

1. Dairy industry in Ethiopia

1.1. Dairy products' consumption

Milk and dairy products are healthy foods and should be safe for consumption. Consumption levels vary enormously around the world (FAO, 2009). The World Health Organization (WHO) indicates a yearly milk consumption of 212 kg per capita in industrialized countries (WHO, 2003). However, Africans consume on average not more than 26 kg milk per year. The average yearly milk consumption in Ethiopia is 19 kg which is even lower than the African average (FAO, 2011). Still, the rapid population growth in Africa in general and in Ethiopia in particular causes a growing demand for milk and dairy products which is only partially answered by a growing production (Mekonnen et al., 2006). Consequently, the gap between the recommended (0.6 kg per day) (https://www.cnpp.usda.gov/dietary-guidelines-2010 accessed on 31 October 2016) and actual milk consumption (19 kg per capita) is widening rather than narrowing.

It is important to mention that milk consumption differs among regions within Ethiopia. In the lowlands, milk is consumed daily by people of all ages (Coppock et al., 1992) whereas in the highlands milk is mainly consumed by children and elderly people (Ahmed et al., 2003). The Central Statistical Agency of Ethiopia (CSA) reported that 42% of the milk produced in rural Ethiopia was self-consumed whereas only 6% was sold (CSA, 2014b). The remaining 52% is processed into products such as butter, fermented milk, and ayib (i.e. resembling cottage cheese) using traditional methods (Figure 1.1) (Gonfa et al., 2001).

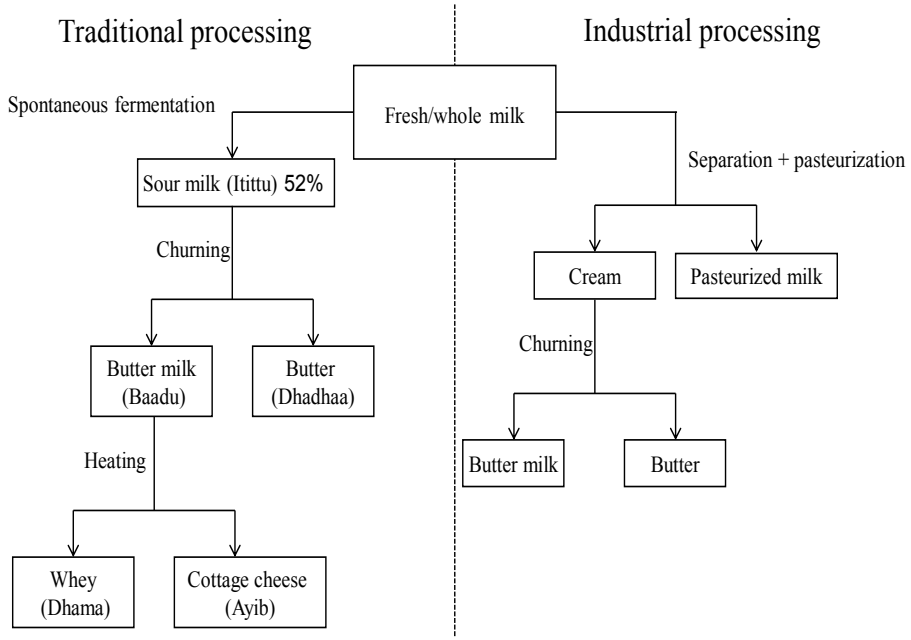

Figure 1.1. Traditional compared to industrial processing of milk in Ethiopia. Adapted from Gonfa et al. (2001).

Most Ethiopian consumers prefer to purchase raw milk because of its natural flavor (high fat content), higher availability and lower price compared to pasteurized milk (Francesconi et al., 2010). However, consumption of raw milk can be a risk for foodborne infections caused by pathogens such as enterotoxigenic *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli) O157:H7, Salmonella* spp.*, Listeria monocytogenes (L. monocytogenes), Campylobacter* spp, *Brucella abortus* (*B. abortus*) and *Mycobacterium bovis* (*M. bovis*) (Radostits et al., 2000; LeBlanc et al., 2006).

1.2. Dairy production

Ethiopia is considered to maintain the largest cattle population in Africa. The CSA estimated the cattle population in rural areas to be about 55 million of which 10.7 million are kept for milking (CSA, 2014a). In rural areas, smallholder farms milk indigenous Zebu breeds under traditional management (Gebrewold et al., 1998; Redda, 2001), grazing from forage in the field with no other supplement. These breeds are characterized by an average lactation period of 6 months and an average milk yield less than 1.5 liters per day and per cow (CSA, 2014a). The rural (traditional) smallholder farms are responsible for 98% of the milk produced and they are non-market oriented. As previously mentioned, most of the milk produced is retained for self-consumption (CSA, 2014b).

Figure 1.2. Rural herd.

Due to urbanization and population growth, more intensive dairy production systems have emerged in or close to cities and are mainly concentrated in and around Addisa Ababa and

regional big cities such as Jimma, Mekele, Bahir Dar, Hawassa, Adama, Dire Dawa, Harar and Asella, (Tegegne et al., 2013). The estimated population of the instensive dairy production systems, including young stock, is 0.6 million (CSA, 2014a). In these so-called "urban smallholder dairy farms", Holstein x Zebu crossbreeds are kept under stall feeding conditions with limited- or zero-grazing systems. Feed supply is not available in sufficient quantities, or when available, with a nutritionally poor quality. Land is property of government, costly to rent and the main constraint to start new farms (personal communication).

Compared to purebred Zebu cows, crossbreeds have a higher genetic merit for milk production. Crossbreed cows produce an average milk yield of 7.8 ± 0.19 liters per day per cow as compared to Zebus (Ayenew et al., 2009). It is estimated that urban smallholder dairy farms contributes 2% of the total milk produced in the country (Ketema and Tsehay, 2004). Nearly in all herds, information such as on calving dates and mastitis records is lacking. Extension and veterinary services are not available. The government's main concern is the rural smallholder dairy farms for which veterinarians are assigned and even paid. The urban smallholder dairy farmers are forced to use smugglers, less trained and non-licensed workers and are extremely dissatisfied with this situation. In contrast to rural smallholder dairy farms, urban smallholder dairy farms are market-oriented and sell most of the milk to retailers or milk processors (Almaw et al., 2008).

Urban smallholder dairy farms in Debre Zeit, a city close to the capital of Ethiopia, sold 64, 15 and 10 % of the produced milk to milk collection centers, retailers and directly to consumers, respectively (Makita et al., 2012), while 10% was self-consumed. However, the information on how the milk chain functions in Jimma and in most of the other growing cities remains unknown. Overall, farms are not registered in Ethiopia. The number of smallholder dairy farms in Jimma fluctuates between 50-66.

Figure 1.3. Urban smallholder dairy farm.

2. Milk quality and safety

2.1. Introduction

Not only the quantity produced but also the quality of milk is of importance. Milk contains many healthy nutrients (Gaucheron, 2011; Claeys et al., 2013), yet when the quality is low, milk is less nutritious and can even threaten consumer health (Jayarao and Henning, 2001). Milk adulteration is one of the most common food frauds obviously lowering the milk quality (Moore et al., 2012).

High-quality milk contains a low number of bacteria and somatic cells and is free of zoonotic pathogens and antimicrobial residues (Oliver et al., 2009). Bacteria present in milk originate from intramammary infection (IMI) or from environmental contamination due to unhygienic farming, and/or milking, and/or handling of the milk (Ashenafi and Beyene, 1994; Jayarao and Henning, 2001; Bonfoh et al., 2006). Fecal milk contamination is often estimated by measuring the number of coliforms (Jayarao et al., 2004). Cooling throughout the milk chain limits the multiplication of bacteria present in the milk. Pathogenic bacteria such as

Campylobacter jejuni (C. jejuni), *Salmonella* spp., *L. monocytogenes*, *E. coli* O157:H7, *B. abortus* and *M. bovis* may be found in milk (Hill et al., 2012) and mainly threaten health of children, pregnant women and elderly (Claeys et al., 2013).

Intramammary infections cause inflammation of the udder (mastitis) characterized by an increase in the concentration of the somatic cells in milk. An elevated composite somatic cell count (SCC) (typically the threshold of 200,000 cells/mL is used to distinguish between infected and non-infected udder quarters) is a proxy for the presence of IMI (Schukken et al., 2003), and negatively associated with nutrient content and shelf-life of milk (Santos et al., 2003; Malek dos Reis et al., 2013). Another risk associated with mastitis is the presence of antimicrobial residues in milk, which is a consequence of not respecting withdrawal periods when treating mastitis (or other infectious diseases) with antimicrobials. They can cause allergic reactions in hypersensitive consumers, interfere with the production of fermented dairy products, and select for antimicrobial resistance (Cogan, 1972; Dewdney et al., 1991; Pereira et al., 2014).

2.2. Milk quality standards

Monitoring of milk quality is essential to ensure healthy milk for human consumption. Milk quality control includes measuring bacterial presence, SCC and detecting antimicrobial residues (Oliver et al., 2009). In the US, so called Grade A milk should conform to the milk quality standards as indicated in the Pasteurized Milk Ordinance (PMO). Accordingly, bulk milk with a total bacterial count (TBC) > 100,000 cfu/mL, SCC > 750,000 cells/mL or positive for drug residues cannot be sold. Similar milk quality standards are used in the European Union (EU). Yet, the EU standard for bulk milk SCC (400,000 cells/mL) is more stringent compared to the US (Hillerton and Berry, 2004). However, guidelines for

monitoring milk quality suggest that when TBC > 10,000 cfu/mL the milk quality is categorized as poor (Jayarao et al., 2004).

In Ethiopia, standards are described for raw milk before processing (QSAE, 2009). Milk can contain up to 3ng/mL penicillin G, 600ng/mL tetracycline, 20ng/mL erythromycin, 10ng/mL streptomycin, 10ng/mL novobiocin, 5ng/mL sulfamethazine, and 80ng/mL chloramphenicol, respectively. Milk with a TBC < 1,000,000 cfu/mL is considered to have a good bacteriological quality. However, the Ethiopian standards do not indicate how to handle milk that does not meet the standards. Neither bulk milk SCC standards nor regular checking of milk quality is available. Yet, legal decisions taken on the people who produce or sell low quality milk is lacking. Also, there are no price differentiations based on milk grades. At present, there is no central laboratory available in Ethiopia, that checks for the milk quality and safety.

2.3. Milk quality tests

Different tests can be used to measure different milk quality parameters. Each test has its advantages and disadvantages. Following tests were used in this PhD research.

Somatic cell count

California Mastitis Test (CMT): A quick, simple test that accurately enumerates the somatic celll count of milk from individual quarters or on composite milk samples or bulk milk samples. CMT reagent is a detergent with a pH indicator (purplish color). When the milk and CMT reagent are mixed in an equal amount, the CMT reagent disrupts the outer cell wall and the nuclear membrane of any leucocytes and the DNA is released from the nuclei. The more DNA is released, the more the mixture thickens. The result is scored and interpreted as either

0, Trace (T), 1, 2 or 3 inflammatory response based on the viscosity of the gel formed by mixing the reagent with milk (NMC, 1999).

Direct Cell Counter (DeLaval, Tumba, Sweden)**:** A milk sample is aspirated in a disposable cassette containing a DNA specific fluorescent reagent. Next, the cassette is placed in a portable device with a digital camera counting the cells within approximately 45 seconds. The results are reliable but the disposable cassettes are relatively expensive (Sarikaya and Bruckmaier, 2006).

Bacterial count

3M™ Petrifilm™ Aerobic Count Plates: The principle of 3M™ Petrifilm™ Aerobic Count Plates (3M, Saint Paul, MN, US) is similar with the standard plate count. However, no agar needs to be made. The ready-to-use plates contain a tetrazolium indicator dye coloring bacteria which facilitates colony enumeration.

3M™ Petrifilm™ Coliform Count Plates (coliform count): Utilizes a petrifilm plate (3M, Saint Paul, MN, US) with a selective medium for coliforms. This test provides a confirmed result in 24 to 48 hours. It reduces the confirmation steps and increases productivity compared with the standard plate count. Besides, it is fast and accurate. However, it is costly and not available in local, Ethiopian markets.

Antimicrobial residues

Copan milk test (Copan Italia, Brescia, Italy)**:** This test contains spores of *Geobacillus stearothermophilus* and nutrients. During incubation with milk, the spores germinate and produce carbonic acid causing a color change of an indicator dye. In case of antimicrobial residues, bacterial growth is inhibited and no color change is observed. This test is simple and reliable for screening of individual cow or bulk milk samples. The test has a high sensitivity

for most veterinary antimicrobials but sensitivity is low for certain antimicrobials such as cefquinome (LeBreton et al., 2007).

Other methods to test milk quality are indicated below but the list is not complete.

Milk adulteration

***Lactometer*:** A lactometer measures the density of a milk sample. Water has a lower density than milk. In case of adulteration, the density will decrease and the lactometer will sink deeper in the fluid (Stone, 1933). This test is quick and simple and applicable at the farm level yet it can be influenced by temperature and fat content.

***Freezing point*:** Similar to the density, the temperature at which water and milk freeze differs. Hence, adulteration can be detected by measuring the freezing point of a milk sample. This technique is more accurate compared to a lactometer but requires a more complex setup (Shipe, 1959).

Somatic cell count

***Direct microscopy cell count*:** The SCC can be estimated by staining cells in a milk sample and counting the number of cells in a given volume under the microscope. This method requires dedication and precision of the lab technician (Brazis et al., 1968).

***Fossomatic™*:** The Fossomatic™ (FOSS Electric A/S, Hillerød, Denmark) is an automated flowcytometer comparable to the Bactoscan™. Yet, instead of bacteria, somatic cells are stained and counted.

Total bacterial count

Methylene blue reduction test: This test is performed by adding methylene blue, a dye losing its color under the absence of oxygen, to milk. As bacteria in milk consume oxygen, the TBC

can be estimated by measuring the time required for the disappearance of the blue color in the milk (Thornton and Hastings, 1930). Although the principle is simple, the test requires skilled manpower and is time consuming.

***Standard plate count*:** The standard plate count (SPC) is performed by pipetting serial dilutions of a milk sample thoroughly spreading over agar in the petri dishes. After 48 h incubation at 32°C, visible colonies are counted (Hayes et al., 2001). This test requires a bacteriology lab and is relatively time consuming.

Laboratory Pasteurized Count: The laboratory pasteurized count (LPC) determines the bacteria that can survive being heated at 62.8 ^{o}C for 30 minutes. LPC is used as an indicator of milking equipment sanitation (Gillespie et al., 2012). Like that of SPC, the LPC test requires a bacteriology lab and is relatively time-consuming.

Preliminary Incubation Count: The preliminary incubation count (PIC) test is used to measure the survival of bacteria in milk when the sample is incubated at 21^{o}C for 18 hours. The PIC is used to measure psychrotrophic bacteria. These bacteria are often associated with reduced shelf life (Gillespie et al., 2012). The PIC test requires a bacteriology lab and is relatively time-consuming.

Bactoscan™: A Bactoscan^{TM} (FOSS Electric A/S, Denmark) measures the bacterial load in a flow cytometer. It allows a rapid, automated and reliable analysis of milk samples (Lachowsky et al., 1997). Yet, the device is expensive.

Antimicrobial residues

Delvotest SP-NT (DSM Food Specialties B.V, Delft, The Netherlands)**:** This test is similar to copan milk test in procedure and application too. The test has a high sensitivity for most

veterinary antimicrobials but sensitivity is low for certain antimicrobials such as cefquinome (LeBreton et al., 2007).

3. Mastitis

3.1. Introduction

Mastitis, an inflammation of the mammary gland, is usually a consequence of a microbial IMI (Watts, 1988; Quinn et al., 1994). The inflammatory response can be visible or invisible. Mastitis with visible symptoms is called clinical mastitis whereas without visible symptoms is called subclinical mastitis (SCM) (FAO, 2014). Clinical mastitis can be recognized by the presence of abnormal milk (discoloration, clots) or swelling of the affected quarter (Gonzalez et al., 1990; Bartlett et al., 1992). Also, the cow can show generalized symptoms such as fever, loss of appetite, reduction in mobility due to the pain of the swollen udder and systemic shock. Subclinical mastitis refers to inflammation of the mammary gland in the absence of visible symptoms. Subclinical mastitis can develop into clinical mastitis and vice versa. Severe or chronic inflammation can result in loss of quarter(s). Cows with blind quarters produce less (Duraes et al., 1982) and are more likely to be prematurely culled than healthy herd mates (Duraes et al., 1982).

3.2. Consequences

Mastitis causes a reduction in milk production (Natzke et al., 1972; Hortet and Seegers, 1998) and detrimental changes in milk quality (Santos et al., 2003) and composition (Hortet and Seegers, 1998). Mastitis is the economically most important disease in the dairy cattle

(Bradley, 2002; Halasa et al., 2007). Clinical mastitis alone costs the UK dairy industry £168 million annually (Bradley, 2002). In Ethiopia, production losses due to SCM were estimated at 38 USD per lactation per cow (Mungube et al., 2005).

The risk for public health should not be overlooked either (Bradley, 2002). The potential spread of foodborne pathogens via milk (Oliver et al., 2009) and the presence of resistant bacterial strains due to extensive use of antimicrobials for mastitis control (Bradley, 2002), and presence of (excess) antimicrobial residues in milk are factors of concern (Petrovski et al., 2006).

Mastitis also threatens animal welfare (Petrovski et al., 2006). Kemp et al. (2008) demonstrated the fact that clinical mastitis causes pain, cows with moderate cases of clinical mastitis had a higher mean rectal temperature, heart rate, and respiratory rate than healthy control cows. Severe cases of mastitis can even result in the death of the affected animal (Petrovski et al., 2006).

Additionally, mastitis treatment is labor intensive reducing the available time of the farmer for other management tasks (Halasa et al., 2007).

Furthermore, discarding mastitic milk and/or milk from treated animal results in substantial food losses, although one should realize that this type of milk is typically not discarded in Ethiopia. Whether this is because the producers are not aware of the associated risks remains to be determined.

3.3. Diagnosis

When diagnosing udder health problems, one needs to distinguish between IMI, clinical mastitis and SCM. Intramammary infections can be detected by performing bacteriological culture or PCR directly on milk samples. In both cases, laboratory equipment is required

(Viguier et al., 2009). The presence of an infectious organism in the mammary gland suggests the establishment of IMI. The definition of IMI is sometimes used interchangeably with SCM (Barkema et al., 1997), which should be avoided.

As aforementioned, clinical mastitis can be detected by palpation of the mammary gland and visual inspection of the milk. The affected quarter can be warm, swollen and/or painful. Milk might have another color, be watery or bloody and may contain clots (Lakew et al., 2009). Since milk seems normal in case of SCM, diagnosing is based on additional testing of milk samples that are taken secundum artem. The SCC can be measured at the quarter, cow and herd level. The California Mastitis Test (CMT) is by far the cheapest test to estimate the SCC and is performed by mixing a milk sample with a detergent dissolving cell walls and releasing DNA. The more DNA is released, the more the mixture thickens. Hence, SCC can be scored based on the degree of thickening. The test is cheap, applicable on farm but comes with inter-operator variation and has a low sensitivity (Leach et al., 2008; Viguier et al., 2009). Besides being a milk quality parameter, bulk milk SCC gives an indication of the prevalence of SCM in the herd (Schukken et al., 2003). Other parameters of inflammation such as N-acetyl-β-D glucosaminidase, lactate dehydrogenase activity and electric conductivity of milk can indicate SCM as well but are less extensively used compared to SCC (Pyorala, 2003).

3.4. Etiology

A variety of microorganisms have been isolated from mastitic milk (Quinn et al., 1994). Mastitis pathogens can be grouped based on their Gram-staining characteristics (Gram-positive or Gram-negative), potential damage they cause to the host (major or minor pathogens) or their epidemiological behavior (contagious or environmental pathogens) (Radostits et al., 2000).

Contagious mastitis pathogens are adapted to survive in/on the host, in particular within the mammary gland, and mainly spread from infected to uninfected udders during milking (Radostits et al., 2000). The cow's environment is the main source of infection for environmental mastitis pathogens. Their concentration can be high in soil, manure, bedding, or contaminated water (Radostits et al., 2000).

Staphylococci, *streptococci* and coliforms are the most common causes of bovine mastitis (Watts, 1988). *Staphylococci* are Gram-positive, catalase-positive cocci and are categorized into coagulase-positive *Staphylococus* spp. (CPS) and coagulase-negative *Staphylococcus* spp. (CNS). *Staphylococcus aureus (S. aureus)* belongs to the subgroups of CPS, and is considered to be a major contagious pathogen (Quinn et al., 1994). The large group of CNS is considered to be minor pathogenic (Bramley and Dodd, 1984). *Streptococcus agalactiae (S. agalactiae)*, *Streptococcus dysgalactiae* (*S. dysgalactiae*) and *Streptococcus uberis (S. uberis)* are the most common streptococcal species causing bovine mastitis (Quinn et al., 1994). All 3 are Gram-positive and catalase-negative major mastitis pathogens. *Streptococcus agalactiae* and *S. uberis* are known as a typical contagious and environmental mastitis pathogen, respectively, whereas *S. dysgalactiae* is equally likely to spread from cow to cow than from the environment to the cow.

Escherichia coli and *Klebsiella* spp. belong to the coliform group and are the most common Gram-negative pathogens causing bovine mastitis. Both are major environmental mastitis pathogens.

The pathogen distribution of clinical mastitis and SCM has been studied in several countries. E.g., *S. uberis* was the most frequently isolated pathogen in clinical mastitis cases on British and Flemish herds (Bradley et al., 2007; Verbeke et al., 2014) whereas *S. aureus* was the most frequently isolated pathogen causing clinical mastitis in Canada and Ireland (Olde Riekerink et al., 2008; Keane et al., 2013). Coagulase-negative *staphylococci* were the

most common cause of SCM cases in the UK and Flanders (Bradley et al., 2007; Piepers et al., 2007). Coagulase-negative staphylococci were the most common bacteriological outcome of SCM cases in Uganda (Abrahmsén et al., 2014).

3.5. Prevalence in Ethiopia

Dairy farms in Ethiopia are not registered and therefore the exact number and distribution is lacking. However, reports indicated that the number is increasing yearly although it does not commensurate with population growth (Yilma et al., 2011). The number of herds, which are indicated below, is retrieved from prevalence studies carried out in different regions. Most studied farms are similar in average herd size, milk production and farming practices. In addition, most farms are hand-milked and cows are managed under zero-grazing conditions. However, the differences observed between studies might be explained by the differences between individual farm management, environment, and breed of the animals.

In Ethiopia, the prevalence of clinical mastitis ranged from 3.2% to 26.5% at the cow level and from 0.9% to 14.9% at the quarter level (Table 1.1). The prevalence of clinical mastitis was the lowest in Selale (Getahun et al., 2008) and the highest in Asella (Lakew et al., 2009). Unlike other countries (e.g. Barkema et al., 1998; Olde Riekerink et al., 2008; Verbeke et al., 2014), no longitudinal studies have been performed on clinical mastitis in Ethiopia. Consequently, the incidence and average duration of clinical mastitis cases are unknown.

The prevalence of SCM ranged from 25.4% to 46.6% at the cow level and from 11.4% to 30.4% at the quarter level (Table 1.1). The lowest and highest quarter prevalence of SCM were observed in Southern Ethiopia (Sodo and Awasa) (Kerro and Tareke, 2003) and Asella (Lakew et al., 2009), respectively. The between-study variation might be attributed to differences in management and environmental conditions in the different study areas as well as cow breed variations in susceptibility. However, differences in the methodology of the

studies might also explain part of the variation. For example, some authors defined SCM as a CMT score ≥1 (Getahun et al., 2008) whereas others only considered quarters with a CMT score ≥2 to suffer from SCM (Haftu et al., 2012). Nevertheless, it can be concluded that comparison to other countries, the prevalence of subclinical (or clinical or both) mastitis is high in Ethiopia (Table 1.1).

Table 1.1. The prevalence of clinical mastitis (CM) and subclinical mastitis (SCM) in different Ethiopian studies

Region	Herd	Cows			Quarters			Reference (s)
	n	n	% CM	% SCM	n	% CM	% SCM	
Sodo and Awassa	20	307	15.0	25.4	1133	7.3	11.4	Kerro Dego and Tareke (2003)
Addis Ababa	51	363	6.6	46.6	1452	2.7	26.7	Mungube et al. (2004)
Selale	109	500	3.2	29.4	2000	0.9	13.2	Getahun et al. (2008)
Asella	42	223	26.5	38.1	892	14.9	30.4	Lakew et al. (2009)
Adama	95	206	6.3	41.7	794	2.4	22.2	Mekonnen and Tesfaye (2010)
Mekelle	13	305	3.6	33.8	1220	14.3	11.9	Haftu et al. (2012)

Workineh et al. (2002) performed bacteriological culture on 396 quarters of cows housed in two large dairy herds in Repi and Debre Zeit and reported an IMI prevalence of 30%. Getahun et al. (2008) reported 7.4% of the cows in Selale (37/500) had at least one blind quarter. In total, 2.3% of the quarters were blind (45/2000).

Figure 1.4. Study areas of different mastitis studies performed in Ethiopia.

The pathogen distribution of CM and SCM has also been studied in different Ethiopian regions (Table 1.2; Figure 1.4). *Escherichia coli* was the most common pathogen causing CM in Addis Ababa (Workineh et al., 2002) and Mekele (Haftu et al., 2012) whereas *S. aureus* was the most common pathogen causing CM in Asella (Lakew et al., 2009). *Staphylococcus aureus* was the most frequently isolated pathogen from SCM samples in Addis Ababa (Workineh et al., 2002), Selale (Getahun et al., 2008) and Mekele (Haftu et al., 2012). *Staphylococci* other than *S. aureus* were more prevalent in the SCM samples in Asella compared to the prevalence of *Staphylococci* other than *S. aureus* in the other regions (Lakew et al., 2009).

Table 1.2. Pathogen distribution (in %) of clinical mastitis (CM) and subclinical mastitis (SCM) samples collected in different Ethiopian regions

Isolates	Regions						
	Addis Ababa[a]		Selale[b]	Asella[c]		Mekele[d]	
	CM	SCM	SCM	CM	SCM	CM	SCM
Staphylococcus aureus	21	50	43	29	20	11	43
Non-*aureus* staphylococci	10	20	24	8	24	4	10
Streptococcus agalactiae	18	2	13	15	11	11	6
Streptococcus dysgalactiae	3	5	2	7	7	11	1
Streptococcus uberis	5	5	10	3	4	0	3
Escherichia coli	23	0	1	14	3	57	19
Klebsiella spp	0	2	0	0	0	7	9
Other	21	16	7	24	31	0	9

[a]Thirty-nine quarters with clinical mastitis were studied (Workineh et al., 2002). Subclinical mastitis (n = 82) was defined as a California Mastitis Test (CMT) score of 1 or higher and a culture positive result.

[b]Subclinical mastitis (n = 195) was defined as a CMT score of 1 or higher (Getahun et al., 2008).

[c]Fifty-nine quarters with clinical mastitis were studied (Lakew et al., 2009). Subclinical mastitis (n = 74) was defined as a CMT score of 1 or higher.

[d]Twenty-eight quarters with clinical mastitis were studied (Haftu et al., 2012). Subclinical mastitis (n = 100) was defined as a CMT score of 2 or higher.

3.6. Risk factors

Mastitis is a multifactorial disease. Identification of (manageable) risk factors and characteristics associated with the likelihood of the disease, can lead to better control of mastitis. Different herd, cow and quarter characteristics associated with (pathogen-specific) IMI or (sub) clinical mastitis have been identified (e.g. Barkema et al., 1998; Zadoks et al., 2001; Piepers et al., 2011). However, as management largely differs between regions, not all associations can be generalized, leaving the need for implementation of region- or even herd-specific control plans.

In Africa, researchers from Zimbabwe, Uganda and Rwanda reported that farms with pure and cross-breed had higher odds to mastitis compared with the indigenous breed (Katsande et

al., 2013; Abrahmsén et al., 2014; Iraguha et al., 2015). Katsande et al. (2013) also reported that farms using pre-milking teat dipping had lower odds to mastitis compared with farms not using pre-milking teat dipping.

In Ethiopia, Kerro Dego and Tareke (2003) identified early lactation stage (1-120 DIM vs. 121-240 DIM), higher level of Holstein blood (vs. Zebu), teat/udder lesions and/or tick infestation (vs. no lesions or tick infestation) and higher parity as risk factors for clinical mastitis and SCM combined (as the outcome variable IMI culture results (pathogen-specific) was not used rather than clinical symptoms and CMT results were used). Almaw et al. (2008) reported a higher prevalence of SCM (defined as a CMT score ≥1) in Zebu-Holstein crossbreeds (vs. pure Zebu), cows in late lactation stage (>210 DIM vs. <121 DIM) and older cows. Poor hygiene of milking, higher level of Holstein-Zebu blood (vs. Arsi), higher parity and teat/udder lesions (vs. lower parity and teat/udder with no lesions) were risk factors for SCM (defined as a CMT score ≥1) (Lakew et al., 2009). Although a number of risk factors were identified in those studies, most are "unmanageable". E.g. the farmer does not really have an influence on the parity or stage of lactation of his cows. So, there is a need to identify management practices that have an effect on mastitis and that are really relevant in the field.

3.7. Mastitis control

Mastitis control includes treatment of existing IMI and prevention of new IMI (Schmidt, 1969). In the 1960s, the *Five-Point Plan* was initiated in the UK (Neave et al., 1969): (1) early detection and treatment of clinical cases, (2) blanket dry cow therapy (treating all cows with antimicrobials at dry-off) (Eberhart, 1986), (3) post milking teat disinfection (Galton et al., 1988), (4) identification and culling of chronically infected cows and (5) the routine maintenance of the milking machine.

Implementation of the five-point plan was mainly successful in controlling contagious mastitis pathogens but less effective against environmental mastitis pathogens (Bradley, 2002). Therefore, an extended 10-point mastitis control plan was designed by the National Mastitis Council (NMC, a global organization for mastitis control and milk quality). This program includes preventive measurements against environmental mastitis pathogens such as maintenance of a clean, dry, comfortable environment. A North American and International version of this program is available online (NMC, 2016). Yet, not all measurements are applicable on Ethiopian farms. For instance, dry cow tubes are not on the market although NMC still advocates blanket dry cow treatment.

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Chapter 2:

Aims of the thesis

Mastitis remains an economically important disease in the dairy industry worldwide. Dairy consumption as well as production systems differs among regions within Ethiopia.

The general aims of this thesis were to investigate milk production, quality and consumption and to study the prevalence of mastitis and associated risk factors at the herd-, cow- and quarter-level in smallholder dairy farms in Jimma.

The specific aims were:

To quantify milk production and marketing chains, milk quality and consumption (**Chapter 3.1**).

To investigate subclinical mastitis and associated risk factors using the California Mastitis Test, a simple cow-side test detecting high concentrations of inflammatory cells in milk (**Chapter 4.1**).

To analyse pathogen group specific risk factors for clinical mastitis, intramammary infection and quarter being blind by observation, palpation and bacteriological culture (**Chapter 4.2**)

In **Chapter 5**, suggestions for future research are made and potential strategies to increase milk production, to improve milk quality and to reduce udder health problems in Jimma and similar cities are discussed.

Chapter 3:

Milk quality, production and consumption

Chapter 3.1.

Milk production, quality, and consumption in Jimma (Ethiopia): facts and producers', retailers', and consumers' perspectives.

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ABSTRACT

Four studies were performed to quantify milk production, quality and consumption in the town Jimma, Ethiopia. First, 47 dairy farmers and 44 milk retailers were interviewed to gain more insights in dairy farming and marketing, and associated constraints. Second, bulk milk samples (n = 188) were collected for 4 consecutive weeks to investigate milk quality [Total Bacterial Counts (TBC), Coliform Counts (CC), Somatic Cell Counts (SCC), and antimicrobial residues]. Third, (bulk) milk samples from 32 farms, 46 milk retailers and the 3 local milk collection centers were collected to determine the presence of oxacillin susceptible–and oxacillin resistant *Staphylococcus aureus*. Fourth, 208 adult inhabitants were interviewed to gain more insight in milk consumption and associated concerns of consumers. The average dairy farm included in the studies consisted of 5 lactating cows, produced 43 liters of milk per day and was owned by male, literate adults. Milk was sold to retailers (71% of the production) and directly to consumers (25%) without any quality control, whereas 4% was self-consumed. Shortage of animal nutrition and adulteration of the milk were the main constraints for farmers and retailers, respectively. The median TBC, CC and SCC were 122,500 cfu/mL, 1,005 cfu/mL and 609,500 cells/mL, respectively. Antimicrobial residues were detected in 20% of all samples. In general, the milk quality was considered to be poor (TBC > 10,000 cfu/mL, and/or CC > 100 cfu/mL, and/or SCC > 400,000 cells/mL and/or presence of antimicrobial residues) in 97% of all samples. *S. aureus* was isolated from 12 (38%), 13 (33%), and 2 out of 3 of the milk samples originating from the dairy farms, the milk retailers, and the milk collection centers, respectively. Seven (26%) of the isolates were resistant to oxacillin suggesting the presence of MRSA (Lee, 2003). Local milk is occasionally consumed by adults but more frequently by children. Adults mainly drink spontaneously fermented milk (57% of 105 interviewees consuming local milk) whereas most

milk for children is boiled (86% of 110 households with children consuming local milk). Most consumers are concerned about adulteration and milkborne diseases but not about antimicrobial residues. Educated consumers (secondary school or higher) were more likely to boil milk for own consumption, to be concerned about antimicrobial residues in milk, to be concerned about milk borne diseases and to be willing to pay more for milk with proven good quality compared to poorly educated consumers.

We conclude that milk quality incentives should be introduced in Jimma, and investments should be made in knowledge transfer, training, milk collection systems and a central milk quality lab.

Key words: Dairy farming, Ethiopia, Jimma, Milk consumption, Milk quality, *Staphylococcus aureus*

1. Introduction

The demand for milk in cities of developing countries increases due to population growth and urbanization (Narrod et al., 2011). In response, smallholder dairy farms are mushrooming in e.g. Jimma and other Ethiopian towns (Mekonnen et al., 2006). Typically, Holstein–Zebu crossbreed dairy cows are milked with limited or no access to pasture (Tolosa et al., 2013). The smallholders are responsible for 98% of the milk produced. The milk flow and supply chain in Ethiopia is quite complex and in many cases still immature in terms of capacity, organization and infrastructure (Yilma et al., 2011). Only a limited proportion of the milk is bought and sold by the 3 local milk collection centers that have been established by the dairy cooperatives in Jimma in 2011. And most of the milk is directly sold to retailers and/or consumers, most often in producer-owned milk shops.

Intramammary infections (IMI) lead to an increase in somatic cell count (SCC), (in) directly affecting milk shelf-life and components (Ma et al., 2000). High-quality milk has a low SCC and bacteria count, and is free of foodborne pathogens and antimicrobial residues (Oliver et al., 2009). Although milk has a high nutritional value (Gaucheron, 2011), it constitutes a good growth medium for bacteria of which some are pathogenic for humans (Jayarao and Henning, 2001). Bacteria in milk originate from shedding cows with IMI, unclean milking practices or improper milk handling (Hayes et al., 2001; Zadoks et al., 2004). Among other pathogens in milk, *Staphylococcus aureus* can cause severe disease in humans, and is difficult to treat in case of the presence of antimicrobial resistance (Xu et al., 2014). Accurate information on milk quality in Jimma and the whole of Ethiopia is scarce.

Western consumers are highly concerned about the quality of milk and other animal products (Aumaître, 1999; Noordhuizen and Metz, 2005). Whether consumers in African cities such as Jimma share this concern is largely unknown. The risk of milkborne diseases does not only depend on microbiological characteristics but also on milk processing before

consumption (Lejeune and Rajala-Schultz, 2009). In Ethiopia, milk is often not pasteurized but consumed after spontaneous fermentation at the household level (Gonfa et al., 2001). Spontaneous fermentation helps in extending the storage life of the milk. During the spontaneous fermentation, the lactic acid bacteria present in the raw milk convert the lactose into lactic acid, lowering the pH of the milk and thus resulting in a reduced bacterial count. Some of the lactic acid bacteria also produce bacteriocins that inhibit the growth of disease-causing pathogens (Gillor et al., 2008). Yet, the frequency and type of milk consumed by adults and children, being more susceptible to milkborne diseases, is unknown in cities such as Jimma.

The objectives of this research were to characterize dairy farming and marketing, to study milk quality, to detect the presence of (oxacillin resistant) *S. aureus* in raw (bulk) milk and to quantify milk consumption in Jimma, Ethiopia.

2. Materials and methods

2.1. Study area

Jimma is a medium-sized town with approximately 140,000 inhabitants located in Oromia Regional State, Jimma Zone, 352 km South-West of the capital, Addis Ababa in Ethiopia. Jimma has an altitude of about 1780 m above the sea level and an annual rain-fall ranging from 1400 to 1900 mm. Temperature varies between 6◦C and 31◦C (Alemu et al., 2011). The area is mainly known for its coffee production but crop and livestock production are important agricultural activities as well.

2.2. Questionnaire on dairy farming, milk production and marketing

As (dairy) farmers and retailers are not registered in Ethiopia, their exact total number is unknown, also in Jimma. Yet, 47 smallholder dairy farmers (total number estimated at 60) and 44 milk retailers were selected based on their willingness to cooperate, encompassing a large majority of those present in Jimma. All were interviewed face-to-face by the first author between July and August 2009. Closed and open questions were asked to gain more insight in dairy farming and marketing, and associated constraints (Table 3.1.1).

In all 47 herds, different types of antimicrobials were used to treat sick animal against different infectious diseases including mastitis. With a cow level prevalence of clinical and subclinical mastitis of 11 and 62%, respectively, and a blind quarter prevalence of 6%, mastitis is one of the most common infectious diseases for which antimicrobials are used (Tolosa et al., 2013; Tolosa et al., 2015). All except from one herd used Lactaclox® (i.e., a combination of ampicillin and cloxacillin) for the intramammary treatment of mastitis. Sixteen (34%), 13 (28%), 10 (21%), and 8 (17%) herds used Lactaclox® and oxytetracycline, Lactaclox®, Lactaclox®, oxytetracycline and penicillin–streptomycin, and Lactaclox® and penicillin–streptomycin, respectively. On 54% of the herds, antimicrobials and other drugs used for the treatment of diseases were prescribed and administered by a veterinarian on the herd, on 24% of the participating herds sick animals were treated at a veterinary clinic in the neighbourhood and 22% of the farmers treated their animals themselves based on their own experiences. Animals suffering from clinical mastitis are treated for a maximum of 3 consecutive days. No precise information was available on the treatment strategies applied for other diseases than mastitis.

Table 3.1.1. Questions asked to 47 dairy farmers and 46 milk retailers on dairy farming and marketing in Jimma (Ethiopia) using a face-to-face questionnaire.

Farmers/retailers	Question
Farmers	Age (in years)
	Gender (male/female)
	Education (elementary school or lower/secondary school or higher)
	Herd size (number of lactating animals)
	Total daily milk production in litres
	Proportion of milk for own consumption, sold directly to cosumers and sold to milk retailers
Both	Average selling price of milk per litre in Birr
	Concerns about antimicrobial residues (yes/no)
	Concerns about milk quality (yes/no)
	Interest in the establishment of a central milk quality laboratory (yes/no)
	Main constraint (open question)

2.3. Longitudinal study on milk quality

Bulk milk samples of the aforementioned dairy farms were aseptically collected once a week for four consecutive times between December 2009 and January 2010. For the enumeration of TBC and CC, serial dilutions (10^{-1},10^{-2}, 10^{-3} and 10^{-4}) were plated on Petrifilm Aerobic Count Plates (3M, Saint Paul, MN, US) and Petrifilm Coliform Count Plates (3M, Saint Paul, MN, US) according to the manufacturer's instructions. After 24 hours of incubation at 37°C, plates were read using a semi-automated colony counter (Stuart, Bibby Sterilin, Stone, UK). The SCC was measured with a DeLaval Direct Cell Counter (DeLaval, Tumba, Sweden). The Copan Milk Test (Copan Italia, Brescia, Italy) was used to determine the presence or absence of antimicrobial residues in the milk samples according to the manufacturer's instructions.

2.4. Cross-sectional study on Staphylococcus aureus occurrence and oxacillin resistance

Additional bulk milk samples were aseptically collected between October 2012 and May 2013 to determine the occurrence of oxacillin resistant - and oxacillin susceptible *S. aureus*. Samples were collected from 32 of the aforementioned 47 dairy farms, 46 milk retailers and the 3 milk collection centers established in Jimma in 2011. Ten µL of milk was plated on blood-esculin agar and incubated aerobically for 24-48 hours at 37°C (National Mastitis Council, 1999). Round colonies with hemolysis were transferred to a new plate. They were identified as *S. aureus* or other bacteria based on Gram-staining, a catalase test, growth on mannitol salt agar, a DNase test, a coagulase test and polymyxin susceptibility. Subsequently, Oxacillin Resistance Screening Agar Base (ORSAB, Oxoid, Basingstoke, England) was used to test for oxacillin resistance (Simor et al., 2001).

2.5. Questionnaire on milk consumption and associated concerns of consumers

A total of 208 adult inhabitants of Jimma were interviewed using closed questions (Table 3.1.2) on milk consumption, using systemic sampling at every 10th house in the main, secondary and tertiary roads in Jimma. The sample size was estimated using the following formula (Dohoo et al., 2003): $n = \frac{Z^2_{\alpha/2}pq}{L^2} = \frac{1.96^2 * 0.50 * 0.50}{0.1^2} = 96$, with n being the number of consumers needed to estimate the concern for milk quality (a major research question), Zα/2 being the 95th percentile of a standard normal distribution, p being a priori estimate of the proportion of consumers concerned about milk quality intuitively set at 0.5, q being 1 – p, and L being the margin of error set at 0.1. As half of the inhabitants were expected to buy milk regularly, the number of interviewees was doubled. Age of the interviewee was recorded.

Table 3.1.2. Closed questions asked to 208 adults on milk consumption in Jimma (Ethiopia) using a face-to-face questionnaire and their answers.

Question	Answer	n	% (95% CI[a])
Gender	Male	82	39 (33-46)
	Female	126	61 (54-67)
Education	Elementary school or lower	60	29 (23-35)
	Secondary school or higher	148	71 (65-77)
How often do you drink local milk?	Daily	29	14 (9-19)
	Weekly	76	37 (30-43)
	Less often	103	50 (43-57)
How do you drink milk?[b]	Raw	12	11 (5-18)
	Fermented	60	57 (48-67)
	Boiled	33	31 (23-40)
How often do your children drink local milk?[c]	Daily	80	55 (47-63)
	Weekly	30	21 (14-27)
	Less often	35	24 (17-31)
How do your children drink milk?[d]	Raw	4	4 (0-8)
	Fermented	11	11 (5-17)
	Boiled	95	86 (80-93)
Are you concerned about milk quality?[e]	Yes	130	87 (81-92)
	No	20	13 (8-19)
Are you concerned about antimicrobial residues?[e]	Yes	24	16 (10-22)
	No	126	84 (78-90)
Are you concerned about adulteration? [e]	Yes	126	84 (78-90)
	No	24	16 (10-22)
Are you concerned about milk borne diseases? [e]	Yes	114	76 (69-83)
	No	36	24 (17-31)
Would you pay more for milk with proven good quality? [e]	Yes	133	89 (84-94)
	No	17	11 (6-16)

[a]95% confidence interval;

[b]Recorded for adults drinking milk daily or weekly.

[c]Recorded for adults having children below age 10 (n = 145).

[d]Recorded for adults having children below age 10 drinking milk daily or weekly.

[e]Recorded for adults drinking milk daily or weekly and/or having children below age 10 drinking milk daily or weekly.

2.6. Statistical analysis

Descriptive statistics were calculated using Microsoft Excel 2010 (Microsoft Corporation, Redmond, Washington, USA). Because TBC and CC were not normally distributed, the median, interquartile range (IQR) and number of measurements above thresholds as suggested by Jayarao et al. (2004) were calculated.

Associations between educational level of the consumer (secondary school or higher vs. elementary school or lower) and answers to the questions on milk consumption were analysed with SAS 9.4 (SAS Institute Inc., NC, USA). Because of low frequencies in some cells, exact logistic regression models were fit. Answers to the questions "How often do you(r children) drink local milk?" were categorized as daily or weekly vs. less often and "How do you(r children) drink local milk?" as boiled vs. raw or fermented. Odds ratio's (OR) and 95% confidence intervals (CI) of the significant associations are reported.

3. Results

3.1. Questionnaire on dairy farming, milk production and marketing

The interviewed dairy farmers were on average 45 years old (range 15-68). Thirty-five (74 %) were male and 30 (64 %) finished secondary school. Average herd size was 5 lactating cows (range 1-23), producing on average 43 litres of milk per day (range 9–203). Some (4%) of the milk produced was self-consumed, 71 % was sold to milk retailers, and 25 % was sold directly to consumers. In 2011, the Jimma town dairy cooperative established 3 milk collection centres that are currently buying and selling a limited proportion of the produced milk (Figure 3.1.1). The milk collection centers test sourness and adulteration of milk using

an alcohol test and lactometer. Quality of milk sold to retailers or directly to consumers is, however, not tested in Jimma. Farmers received an average of 6.1 Ethiopian Birr (1 Ethiopian Birr = 0.05 $) per litre (range 4-8) whereas the milk retailers charged 12.7 Ethiopian Birr per litre (range 8-21). Forty-four (94 %) of the farmers were concerned about the presence of antimicrobial residues in milk whereas only 8 (18 %) of the milk retailers were. All dairy farmers and 41 (93 %) milk retailers were concerned about the milk quality and all were interested in the establishment of a central milk quality lab. Low demand of milk during fasting (30 %), shortage of animal nutrition (23 %) and low milk price (15%) were the main constraints for the dairy farmers whereas adulteration and poor quality of supplied milk (23 and 20 %, respectively) were the main constraints for the milk retailers (Table 3.1.3).

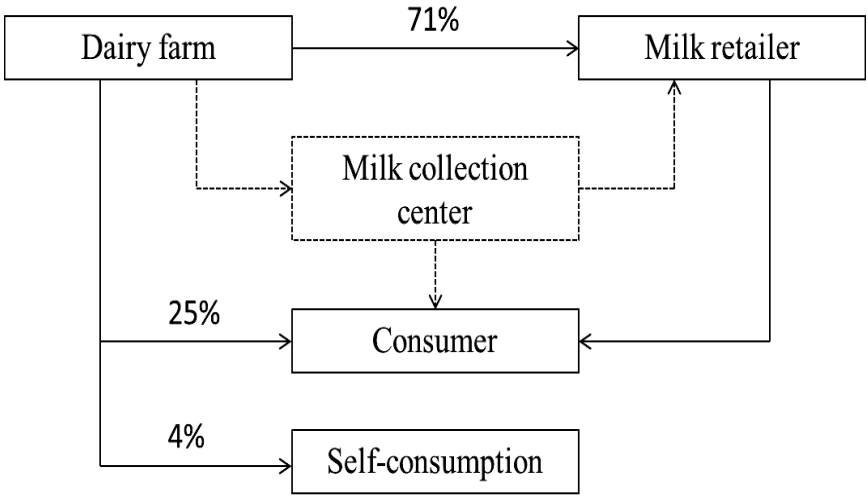

Figure 3.1.1. The raw milk chain in Jimma, Ethiopia. Percentages of production sold to milk retailers, sold directly to customers and self-consumed in 2009 are indicated. The milk collection centers were established in 2011.

Table 3.1.3. Main constraints faced by 47 dairy farmers and 44 milk retailers in Jimma (Ethiopia) as mentioned during a face-to-face interview (open question)

Constraints	Dairy farmers	Milk retailers
No constraints	13 (28%)[a]	12 (27%)
Low demand for milk during fasting	14 (30%)	4 (14%)
Shortage of feed	11 (23%)	-
Low milk price	7 (15%)	-
Adulteration of supplied milk	-	10 (23%)
Poor quality of supplied milk	-	9 (20%)
Shortage of milk supply	-	3 (7%)
Other constraints	2 (4%)	4 (9%)

[a] % between brackets indicates proportion of farmers and retailers, respectively

3.2. Longitudinal study on milk quality

The median TBC, CC and SCC were 122,500 cfu/mL (IQR 12,700-2,850,000), 1,005 cfu/mL (IQR 0-22,750) and 609,500 cells/mL (range 300,000-1,003,000), respectively. Antimicrobial residues were detected in 38 samples (20 %) and on 26 farms (55 %). The milk quality was considered to be poor (TBC > 10,000 cfu/mL and/or CC > 100 cfu/mL and/or SCC > 400,000 cells/mL and/or antimicrobial residues) in 182 of the total number of samples (97 %). The proportion of herds with 0, 1, 2, 3 or 4 milk samples exceeding milk quality thresholds are presented in Table 3.1.4.

Table 3.1.4. Bulk milk quality of 47 dairy farms in Jimma (Ethiopia) measured in 4 consecutive samples

Quality parameter	Threshold	Samples > threshold (%)	% herds with 0, 1, 2, 3 or 4 samples > threshold				
			0	1	2	3	4
Total bacterial count	5,000 cfu/mL	165 (88)	0	0	6	36	57
	10,000 cfu/mL	145 (77)	0	4	21	36	38
Coliform count	50 cfu/mL	133 (71)	0	19	19	21	40
	100 cfu/mL	132 (70)	0	19	19	23	38
Somatic cell count	200,000 cells/mL	159 (85)	0	4	11	28	57
	400,000 cells/mL	120 (64)	4	19	21	28	28
Antimicrobial residues	Detected	38 (20)	45	32	21	2	0
Medium - poor quality milk	Combination[a]	184 (98)	0	0	0	9	91
Poor quality milk	Combination[b]	182 (97)	0	0	0	13	87

[a]Total bacterial count > 5.000 cfu/mL, coliform count > 50 cfu/mL, somatic cell count > 200.000 cells/mL or antimicrobial residues detected.

[b]Total bacterial count > 10,000 cfu/mL, coliform count > 100 cfu/mL, somatic cell count > 400,000 cells/mL or antimicrobial residues detected.

3.3. Cross-sectional study on Staphylococcus aureus occurrence and oxacillin resistance

Staphylococcus aureus was isolated from 12 (38 %) of the milk samples taken from the dairy farms, 13 (33 %) of the samples taken from the milk retailers and 2 (67%) of the samples taken from the milk collection centres. Seven (26 %) of the isolates were resistant to oxacillin.

3.4. Questionnaire on milk consumption and associated concerns of consumers

The interviewees were on average 37 years old (range 18-80). Eighty-two (39%) were male and 148 (71%) finished secondary school. Of the 105 interviewees consuming local milk daily or weekly, 11, 57 and 31% mainly consumed raw, fermented and boiled milk, respectively. Of the 145 interviewees having children below the age of 10, 110 gave local milk to their children daily or weekly. In 4, 11 and 86% of the households, children mainly consumed raw, fermented and boiled milk, respectively. Of the 150 interviewees drinking milk daily or weekly and/or having children below age 10 drinking milk daily or weekly, 87, 16, 84 and 76% were concerned about milk quality, antimicrobial residues, adulteration and milkborne diseases, respectively. Eighty-nine % would pay more for milk with proven good quality (Table 3.1.5). Educated consumers (secondary school or higher) were more likely to boil milk for their own consumption [OR with 95% CI: 6.91 (1.51-64.27)], to be concerned about antimicrobial residues in the milk [OR: 9.15 (1.37-389.33)], to be concerned about milkborne diseases [OR: 5.87 (2.40-14.73)] and to be willing to pay more for milk with proven good quality [OR: 14.39 (4.01-65.94)] compared to poorly educated consumers (Table 3.1.5).

Table 3.1.5. Associations between level of education and answers to closed questions asked to 208 adults on milk consumption in Jimma (Ethiopia) using a face-to-face questionnaire..

Question	Percentage		OR (95% CI)[a]
	Low education[b]	High education[c]	
Boiling of milk before consumption[d]	8	39	6.86 (1.51-64.27)
Concerned about antimicrobial residues[c]	3	20	9.15 (1.37-389.33)
Concerned about milk borne diseases[c]	49	85	5.87 (2.40-14.73)
Willing to pay more for milk with proven good quality[c]	65	96	14.39 (4.01-65.94)

[a]Odds ratio with 95 % confidence interval.

[b]Elementary school or lower.

[c]Secondary school or higher.

[d]Recorded for 24 lowly educated and 80 highly educated adults drinking milk daily or weekly.

[c]Recorded for 37 lowly educated and 113 highly educated adults drinking milk daily or weekly and/or having children below age 10 drinking milk daily or weekly.

4. Discussion

Four studies were performed to gain more insights in dairy farming and milk quality in Jimma (Ethiopia). Unfortunately, as farms are not registered and the total number of dairy farms in Jimma is unknown, we cannot be sure the sample is fully representative. Yet, we believe a large majority of farmers has been included.

The majority of farmers in Jimma is literate, male elders. Although dairy herds are relatively small, employees milk the cows rather than the owners (Tolosa et al., 2013). The milk chain in Jimma is decentralized with limited to no quality control, which is clearly reflected in the milk quality results. Surprisingly, most farmers but few retailers declared to be concerned about antimicrobial residues. Yet, milk adulteration and poor milk quality were identified as the main constraints faced by retailers demonstrating the need for more milk quality control in Jimma. On-farm testing of the milk composition via a lactometer or measuring the freezing point via a cryoscope before the milk is sold to the retailers and/or consumers would certainly lower the prevalence of adulteration though is at this moment not realistic due to a lack of infrastructure and resources.

Milk quality was found to be very poor in Jimma. The high TBC and CC suggest unhygienic practice around milking whereas implementation of hygienic measurements such as proper cleaning and disinfection of the milk containers and cooling throughout the milk chain could help to improve the milk quality (Bonfoh et al., 2006). The availability of a range of high quality branded pre-milking and post-milking teat dips would make a significant difference. Also, some farmers do have electricity which could be used to run a small bulk milk tank, though these are the exceptions. Some try to keep the milk cool after milking by

placing the milk bucket or churn in water in an attempt to ensure the milk does not spoil. Of course, particularly in the heat of the summer this is less likely to be successful in keeping the bacterial growth low enough. The high SCC is in accordance with the previously reported high prevalence of subclinical mastitis in Jimma (Tolosa et al., 2013 and 2015). Although most farmers declared to be concerned about antimicrobial residues, 20 % of the bulk milk samples and more than half of the farms tested positive indicating many farmers do not respect withdrawal times, or are not aware of the concept. As the Copan Milk Test has a low sensitivity for certain antimicrobials (Le Breton et al., 2007) and farmers were aware of the sampling taking place, the actual occurrence of antimicrobial residues might be even higher. Routine antimicrobial testing of the milk is not realistic due to costs and time. The often small volumes of milk do generally not add up the economics of carrying out such antimicrobial residue tests. Still, the risk of residue failures can be drastically minimized by a good identification and marking of all treated cows before treatment is administered, by the use of a standardized treatment protocol, and by milking all treated cows last or separately. Providing a written guide on how to avoid antimicrobial residues in the milk on every dairy farm could be a first step towards a lower number of residue failures. The availability of a range of licensed veterinary medicinal products could also make it easier to predict and respect the correct withdrawal period and thus to reduce the number of residue failures.

Most retailers bought milk from one specific farm, most likely explaining why the occurrence of *S. aureus* differed little between samples from farms (38%) and retailers (33 %). A similar occurrence of *S. aureus* (43.5 %) was reported in smallholder dairy farms in Debre Zeit, close to the Ethiopian capital (Makita et al., 2012). A relatively high proportion of isolates was resistant to oxacillin suggesting the presence of MRSA (Lee, 2003). Detection of enterotoxin and resistance genes could increase our knowledge on the risk of food poisoning and resistance mechanisms (Silveira-Filho et al., 2014) and remain to be studied.

Children consume milk more often than adults. Most but not all interviewees declared to boil milk prior to giving it to children. Given the high bacterial load in the milk and the high susceptibility of children to milkborne diseases (American Academy of Pediatrics, 2014), consumption of raw milk should be discouraged in Jimma. Many adults drink spontaneously fermented milk. Yet, under laboratory conditions, pathogens such as *Listeria monocytogenes* and *Escherichia coli* O157:H7 survived spontaneous fermentation (Ashenafi, 1994; Tsegaye and Ashenafi, 2005). The prevalence of the latter pathogens in spontaneously fermented milk might be high but remains to be studied.

Very low concentrations of antibiotics can select for antimicrobial resistance (Huttner et al., 2013), being a threat for global health (Gullberg et al., 2011). Similar to the retailers, few of the consumers were concerned about antimicrobial residues. However, the high concern about milk adulteration and milkborne diseases indicate the need for more milk quality control. As many consumers were willing to pay more for milk with proven high quality, producers could benefit from more milk quality control. Differences with highly educated consumers indicate a lack of knowledge on the risks associated with antimicrobial residues and milkborne diseases in poorly educated consumers.

5. Conclusions

Jimma has a decentralized milk chain with limited to no quality control. Milk quality was found to be poor in all 47 sampled farms. *S. aureus* was frequently isolated with many strains showing oxacillin resistance. The majority of the adults drink unpasteurized milk whereas most but not all children drink pasteurized milk. Consumers are concerned about adulteration and milkborne diseases but not about antimicrobial residues. Based on the results, milk quality incentives should be introduced in Jimma.

Conflict of interest statement

No conflict of interest exists for any of the authors.

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Chapter 4:

Risk factors for mastitis

Chapter 4.1

Risk factors associated with subclinical mastitis as detected by California Mastitis Test in smallholder dairy farms in Jimma, Ethiopia using multilevel modelling

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ABSTRACT

The prevalence of subclinical mastitis and associated risk factors at the herd, cow and quarter level were studied using multilevel modelling in smallholder dairy farms in Jimma, Ethiopia. Forty-two herds, out of the 55 dairy farms located in Jimma (76%), were visited, a questionnaire was performed, and 635 quarters belonging to 176 lactating cows were screened to detect the presence of subclinical mastitis using the California Mastitis Test (CMT). Sixty-two % of the cows and 51% of the quarters were subclinically infected. Overall, quarters from cows in later stage of lactation (>180 DIM) [opposed to early lactation (<90 DIM)] and quarters from cows with a tick-infested udder had higher odds of subclinical mastitis, as reflected by the CMT test. Also, quarters from cows without udder tick infestations milked by squeezing were less likely to be infected than when milked by stripping. However, the milking technique did not influence the odds of infection in cows with ticks on the udder. This study stresses the high prevalence of subclinical mastitis in smallholder dairy farms in Jimma and a lack of awareness of the existence of the disease among dairy farmers. Implementation of a mastitis prevention program adapted to the local needs, including a focus on application of appropriate tick control measurements as well as fertility management, allowing cows to be dried-off at a more appropriate moment, are needed. To conclude, milking by squeezing instead of stripping in particular in cows without udder tick infestations, a better control of tick infestations as well as the implementation of a more appropriate fertility management could reduce the subclinical mastitis prevalence (and incidence) on the short term.

Keywords: Risk factors, Multilevel modelling, Subclinical mastitis, Smallholder dairy farms, Jimma town, Ethiopia, California Mastitis Test

1. Introduction

Ethiopia maintains the largest cattle population in Africa, recently estimated by the Central Statistical Agency of Ethiopia (CSA, 2012) at 52.1 million animals of which 7.2 million are primary held for milk production. Despite these large numbers, milk production lags behind the demand. On average, yearly consumption of milk in Ethiopia is as low as 17 kg *per capita* whereas the average figure for Africa was 26 kg *per capita* in 1998 (Gebrewold et al., 1998). In response to the increasing demand for milk, smallholder dairy farms emerged in urban and peri-urban areas (Mekonnen et al., 2006) such as Addis Ababa and other cities including Jimma, located in South-West Ethiopia.

Mastitis is highly prevalent in different dairy production systems in Ethiopia and largely impairs production (Almaw et al., 2008; Getahun et al., 2008; Lakew et al., 2009; Tolosa et al., 2009; Tesfaye et al., 2010). With the increasing level of the more susceptible Holstein blood in crossbreeds, the disease seems to be becoming more important (Almaw et al., 2008). Subclinical mastitis is, from an economical point of view, considered as the most important type of mastitis because of the higher prevalence and devastating long term effects of chronic infections compared to clinical mastitis. Production losses in Ethiopian crossbreeds due to subclinical mastitis have been estimated at 38 USD per lactation per cow (Mungube et al., 2005). However, because of lack of clinical symptoms and a quality control system, few farmers in Jimma and other parts of the country are aware of the subclinical form of mastitis and its consequences.

Knowing the prevalence of and identifying risk factors associated with subclinical mastitis is essential in designing prevention and control measures against the disease. However, availability of this information is limited in Jimma and other parts of Ethiopia, mainly because of lack of laboratory capacity. Therefore, the objectives of this study were to estimate the

prevalence of subclinical mastitis at herd, cow and quarter level in smallholder dairy farms in Jimma town using the California Mastitis Test, and to identify associated risk factors as a first step toward increasing knowledge of the disease occurrence and potential control mechanisms.

2. Materials and methods

2.1. Description of the study area

Jimma is a medium-sized Ethiopian town with approximately 140,000 inhabitants located in Oromia Regional State, Jimma Zone, 352 km South-West of the capital, Addis Ababa. The town's geographical coordinates are approximately 7°40' N latitude and 36° 50'E longitude. Jimma has an altitude of about 1780 m above sea level and an annual rainfall ranging from 1400 to 1900 mm. Temperature varies between 6°C and 31°C (Alemu et al., 2011). The area is mainly known for its coffee production but crop and livestock production are important agricultural activities as well. Milk is produced in small dairy farms established in the city and sold to milk retailers and/or to consumers, most often in producer-owned milk shops.

2.2. Study herds and animals

Forty-two dairy herds of Jimma town dairy cooperative, out of the 55 active dairy herds in Jimma, were visited between July 2010 to June 2011 by a member of a team consisting of two veterinarians and two technicians. Herds were conveniently selected based upon motivation of the farmers. On average, four cows were milked per herd, ranging between one and ten (Table 4.1.1). Cows were tethered and hand-milked using the whole hand (five finger squeezing) or

only thumb, index and middle finger (stripping) by employees or owners. Lack of space enforces most farms to strictly practice zero-grazing. Neither blanket dry cow therapy nor teat spraying or dipping after milking is performed. Calves are either bucket-fed or suckle the cows.

Table 4.1.1. Hierarchy of the dataset and descriptive statistics of number of herds, cows, and quarters included in the study.

Level	Total number	Average number at next higher level	Range at next higher level
Herd	42	…….	…….
Cow	176	4.2	1-10[a]
Quarter	635	3.6	1-4

[a]Referring to the number of lactating cows

2.3. Data collection

During the farm visit, potential risk factors for subclinical mastitis at the herd, cow and quarter level were recorded through interviewing of the owner and by observation. Milk samples of all lactating cows (n = 176) were collected (Table 4.1.1):

Herd level information - Information on herd size (two categories: ≤ 10 versus > 10, including lactating cows, heifers, bulls and calves), calf feeding (bucket-fed versus suckling), and number of milking personnel (employees, family members; three categories: ≤ 3, 4-6, > 6 milkers) was recorded. Milking technique and hygiene was studied in more detail; questions on hand washing before milking (yes versus no), washing of the udder before milking (no, only the teats, the whole udder), teat drying before milking (no drying; yes by using 1 towel for multiple cows; yes by using 1 towel per cow), milking technique (squeezing versus stripping) and milking cows with clinical mastitis last (yes versus no) were asked. Data on housing were noted as well including subjects as grazing (zero-grazing versus limited access

to pasture), floor type (solid concrete versus wood/soil) and straw/sawdust bedding (yes versus no) (Table 4.1.2a).

Cow level information - Age (2 categories: ≤ 4 y versus > 4 y), parity (primiparous versus multiparous) and lactation stage [3 categories: < 90 days in milk (DIM), 90-180 DIM, > 180 DIM] was recorded for every cow. Body condition score (BCS; 5 categories: 1 to 5) of all enrolled cows was measured as described by Edmondson et al. (1989). Udder and leg hygiene was scored using a four point scoring system (4 categories: 1 to 4) as described by Schreiner and Ruegg (2002). Flank hygiene was scored using the same definitions. Cows were clinically examined for presence of tick infestation on the udder (yes versus no).

Quarter level information - For each quarter, position (4 categories: left front, left hind, right hind, right front) and presence of teat lesions (2 categories: yes versus no) were recorded.

2.4. Subclinical mastitis

Subclinical mastitis was diagnosed by performing the California Mastitis Test (CMT) on all lactating, clinically healthy quarters. Procedures and interpretations described by the National Mastitis Council (1999) were followed. The first stream of milk was discarded and then a few streams of milk were collected in the corresponding paddle wells. The paddle was tilted to remove excess of milk and an equal amount of a commercial reagent (DeLaval mastitis test CMT, DeLaval operations, Poland) was added to each cup. A gentle circular motion was applied in a horizontal plane for 15 seconds to mix milk with reagent. The result was scored and interpreted as either 0, Trace (T), 1, 2 or 3 inflammatory response based on the viscosity of the gel formed by mixing the reagent with milk.

Table 4.1.2a. Descriptive statistics on subclinical mastitis at the quarter level as reflected by the California Mastitis Test (CMT) and univariable associations with herd characteristics in 42 smallholder dairy herds in Jimma town, Ethiopia using multilevel modelling.

Herd level risk factors	N herds	N lactating quarters		P-value[a]
		N included	Subclinically infected (%)	
Herd size[b]				0.830
≤ 10 animals	19	183	95 (52)	
> 10 animals	23	452	231 (51)	
Calf feeding				0.791
Bucket-fed	23	379	189 (50)	
Suckled	19	256	137 (54)	
Milking personnel				0.407
Family member(s) only	6	80	48 (60)	
External employee(s)	36	555	278 (50)	
People working on farm				0.373
≤ 3	34	464	246 (53)	
3-6	6	134	64 (48)	
> 6	2	37	16 (43)	
Hand washing before milking				0.821
No	15	236	126 (53)	
Yes	27	399	200 (50)	
Washing udder before milking				0.592
No washing	2	59	32 (54)	
Teats only	17	247	120 (49)	
Whole udder	23	329	172 (52)	
Teat drying before milking				0.537
No drying	31	483	240 (50)	
Yes, one towel for multiple cows	6	98	54 (55)	
Yes, one towel per cow	5	54	32 (59)	
Milking technique				0.008
Stripping[c]	29	403	230 (57)	
Squeezing[d]	13	232	96 (41)	
Milking cows with clinical mastitis as last				0.439
No	25	407	216 (53)	
Yes	17	228	110 (48)	
Grazing type				0.933
Zero-grazing	35	534	272 (51)	
Limited access to pasture	7	101	54 (53)	
Stable floor type				0.445
Concrete	30	508	253 (50)	
Wood or Soil	12	127	73 (57)	
Straw or sawdust bedding in use				0.360
No	30	352	172 (49)	
Yes	12	283	154 (54)	

[a]Overall P-value for association of risk factors with outcome variable
[b]Including lactating cows, heifers, bulls, and calves
[c]Hand-milked using only thumb, index and middle finger
[d]Hand-milked using the whole hand (all fingers)

Quarters with CMT score T, 1, 2, or 3 were considered as subclinically infected (1) while those with CMT score 0 were considered as non-infected (0). A cow was considered to have subclinical mastitis if it had at least one subclinically infected quarter.

2.5. Statistical analysis

Associations between the subclinical infection status (0, non-infected versus 1, subclinically infected) of the quarters as outcome variable and potential herd, cow and quarter risk factors were determined using logistic mixed regression models with herd and cow as random effects to correct for clustering of cows within herds and quarters within cows (MlwiN 2.02, Centre for Multilevel Modelling, Bristol, UK). A stepwise model building approach as described by De Vliegher and colleagues (2004) was followed and odds ratio's (OR) with 95% Confidence Intervals (95% CI) were calculated.

In a first step, univariable associations were analysed between the binary outcome variable and all independent variables. Statistical significance in this step was assessed at $P < 0.15$. Next, Spearman Rank correlation coefficients between significant independent variables (n = 6) were calculated using SPSS software version 19 (Chicago, IL, USA) to avoid multicollinearity. If two factors had a correlation coefficient > 0.6, only one was further included in the multivariable analysis. In this case, age was found to be highly correlated with parity ($R = 0.78$) and omitted from further analysis. Finally, the remaining four independent variables were fit as fixed effects in a multivariable model. Non-significant variables were removed using backwards stepwise elimination at $P < 0.05$ and confounding was checked for:

a variable was considered to act as a confounder if its removal made the regression coefficients of the remaining variables undergo a relative change > 25% or in case the regression coefficient ranged between -0.4 and 0.4, if an absolute change of > 0.1 was observed (Noordhuizen et al., 2001). No confounders were detected in this way. Finally two-way interactions between the remaining risk factors in the final model were calculated and tested at $P < 0.05$ (Table 4.1.3).

Table 4.1.2b. Descriptive statistics on subclinical mastitis at the quarter level as reflected by the California Mastitis Test (CMT) and univariable associations with cow characteristics in 42 smallholder dairy herds in Jimma town, Ethiopia using multilevel modelling.

Cow level risk factors	N cows	N lactating quarters		P-value[a]
		N examined	Subclinically infected (%)	
Age in years[b]				0.023
≤ 4 years	50	193	81 (42)	
> 4 years	126	442	245 (55)	
Parity[b]				0.096
Primiparous	52	199	88 (44)	
Multiparous	124	436	238 (55)	
Lactation stage				0.009
< 90 DIM[c]	48	177	73 (41)	
90-180 DIM	53	194	93 (48)	
> 180 DIM	75	264	160 (61)	
Body condition score[d]				0.315
Score 1	9	32	22 (69)	
Score 2	14	53	32 (60)	
Score 3	61	218	102 (47)	
Score 4	63	230	122 (53)	
Score 5	29	102	48 (47)	
Udder hygiene score[e]				0.399
Score 1	40	571	304 (53)	
Score 2-4	136	64	22 (34)	
Flank hygiene score[e]				0.399
Score 1	10	34	15 (44)	
Score 2-4	166	601	311 (52)	
Leg hygiene score[e]				0.396
Score 1	5	17	7 (41)	
Score 2-4	171	618	319 (52)	
Tick infestation of the udder				0.001
No	156	564	271 (48)	
Yes	20	71	55 (77)	

[a] Overall P-value for association of risk factors with outcome variable

[b] Age was highly correlated with parity (R = 0.78) and omitted from further analysis

[c] Days in milk

[d] As in Edmonson *et al*. (1989)

[e] 1 = completely free of dirt or has very little dirt; 2 = slightly dirty; 3 = mostly covered in dirt; or 4 = completely covered in dirt.

The fit of the final model was evaluated by inspection of the cow and quarter level standardized residuals plotted against the normal scores and against the cow and quarter level

predicted values, respectively. The Hosmer-Lemeshow goodness-of-fit test was assessed on the fixed effects models (Dohoo et al., 2009). The test was not statistically significant (P = 0.983).

The proportion of variation in the outcome variable at the herd, cow and quarter level was estimated by assuming that the variance at the quarter level on the logit scale was $\pi^2/3$ (Dohoo et al., 2001) as we have applied before using similar data on quarter level intramammary infection (Piepers et al., 2011).

3. Results

3.1. Descriptive statistics

Not all 176 investigated cows had 4 functional quarters. Forty-three quarters (6.1%) could not be milked; 26, 7 and 1 cow(s) had 1, 2 or 3 blind quarter(s), respectively. Twenty-six quarters belonging to 20 cows had clinical mastitis at the time of sampling and were omitted from the analysis. From the remaining 635 quarters, 326 (51.3%) quarters were considered subclinically infected. Hundred-and-nine (61.9%) cows suffered from subclinical infection. Descriptive statistics by herd, cow and quarter characteristics are summarized in Table 4.1.2a, b, c.

Table 4.1.2c. Descriptive statistics on subclinical mastitis at the quarter level as reflected by the California Mastitis Test (CMT) and univariable associations with quarter characteristics in 42 smallholder dairy herds in Jimma town, Ethiopia using multilevel modelling

Quarter level risk factors	N lactating quarters		P-value[a]
	N examined	Subclinically infected (%)	
Quarter position			0.880
Left front	155	77 (50)	
Left hind	158	80 (51)	
Right hind	159	81 (51)	
Right front	163	88 (54)	
Teat injury			…[b]
No	619	310 (50)	
Yes	16	16 (100)	

[a] Overall P-value for association of risk factors with outcome variable

[b] Convergence not reached

Table 4.1.3. Final multilevel, multivariable logistic regression model describing the association between herd and cow level risk factors and subclinical mastitis at the quarter level as reflected by the California Mastitis Test in 42 smallholder dairy herds in Jimma town, Ethiopia.

Independent variable	β[a]	SE[b]	OR[c]	95% CI[d]	P-value[e]
Intercept variable	-1.029	0.255	…	…	…
Milking technique (herd)					NS[j]
Stripping[g]	Ref[f]				
Squeezing[h]	-0.800	0.231	0.45	0.29-0.71	
Lactation status (cow)					0.002
≤ 90 DIM[i]	Ref				
>90-180 DIM	0.274	0.277	1.32	0.76-2.26	
>180 DIM	0.875	0.259	2.40	1.44-3.99	
Tick infestation of the udder (cow)					<0.001
No	Ref				
Yes	0.710	0.427	2.03	0.88-4.70	
Milking technique x tick infestation[k]					0.016

[a] Regression coefficient

[b] Standard error of the mean

[c] Odds ratio

[d] 95% confidence interval

[e] P-value for overall effect

[f] Reference

[g] Hand-milked using only thumb, index and middle finger

[h] Hand-milked using the whole hand (all fingers)

[i] Days in milk

[j] Non-significant

[k] Interaction term between "Milking technique" and "Tick infestation" - for estimates, see Fig.4.1.1.

3.2. Risk factors for subclinical mastitis

The univariable analysis revealed 1 herd- and 3 cow-level risk factors to be significantly ($P < 0.15$) associated with subclinical mastitis at the quarter level (Table 4.1.2a, b, c). Two significant risk factors remained in the final multilevel, multivariable logistic regression model (lactation stage, tick infestation) as well as the interaction term between milking technique and tick infestation (Table 4.1.3). Quarters from cows in later stage of lactation (>180 DIM) [opposed to early lactation, (<90 DIM)] were more likely to be subclinically infected (OR = 2.40, 95% CI = 1.44-3.99). Quarters from cows with a tick infested udder were more likely to be subclinically infected (OR = 2.03, 95% CI = 0.88-4.70) than quarters of cows without ticks on the udder independently from the milking technique. The overall association milking technique and the likelihood of infection was not significant, but did depend on whether the udder was tick-infested or not (i.e. significant interaction term between 'milking technique' and 'tick infestation', as correctly stated in Table 4.1.3). Quarters from cows without udder tick infestation milked by squeezing were less likely to be subclinically infected than when milked by stripping (OR = 0.45, 95% CI = 0.29-0.71). Still, the milking technique did not influence the likelihood of infection in cows with ticks on the udder (Fig. 4.1.1).

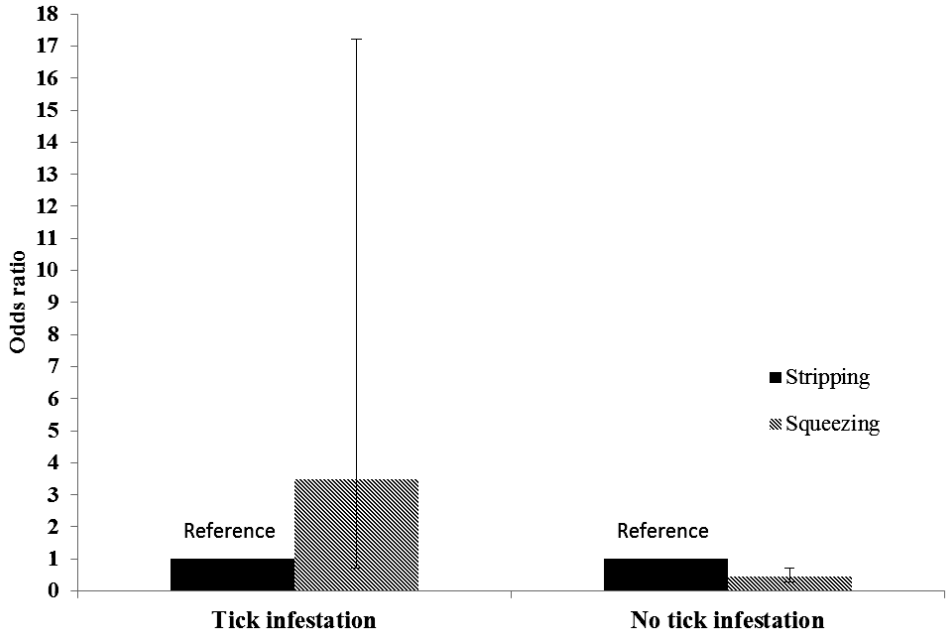

Figure 4.1.1. Interaction term between "Milking technique" and "Tick infestation" visualised using odds ratio's and 95% confidence intervals.

The variance components of the null-model (intercept only) and final multivariable model are presented in Table 4.1.4. In the null-model, 4.1, 16.0 and 79.8% of the variation resided at the herd, cow and quarter level, respectively. These proportions only changed slightly in the final multivariable model. Of the total variance of the outcome variable, only 3.4% of the variation was explained by the variables included in the final model. Interestingly, the variation present at the herd level was entirely explained by the fixed effects included in the final multivariable model.

Table 4.1.4. Variance components at the herd, cow and quarter level of the null and final multivariable model for subclinical mastitis at the quarter level as reflected by the California Mastitis Test in 42 smallholder dairy herds in Jimma town, Ethiopia.

Data hierarchy	Null model		Final multivariable model	
	Var. Est.[a] ± SE[b]	%	Var. Est. ± SE	%
Herd	0.17 ± 0.14	4.1	0.00	0.00
Cow	0.66 ± 0.21	16.0	0.69 ± 0.21	17.3
Quarter	3.29	79.8	3.29	82.7
Total Variance	4.12	100.0	3.98	100.0

[a] Variance estimate

[b] Standard error

4. Discussion

The objectives of this study were to estimate the prevalence of subclinical mastitis using CMT and to identify associated risk factors in smallholder dairy farms in Jimma town, Ethiopia, at the herd, cow, and quarter level. This was done using multilevel modelling to account for clustering of quarters within cows and quarters within herds whereas others, e.g. Mungube et al. (2004) ignored clustering in their data. The latter potentially has had an influence on the estimates (OR's) and might have resulted in overall lower P-values (Dohoo et al., 2009). Our study used data from a substantial proportion of the smallholder dairy farms active in Jimma town, all members of the local dairy cooperative. Because these farmers manage the same breed of cows under the same conditions and in a comparable way as the farmers that are not a member of the cooperative we believe that the results are still valid for all small-holder dairy farms in Jimma. Still, we cannot completely rule out the fact that some selection bias might have been introduced making extrapolation of the results towards all farms in Jimma somewhat more difficult.

Previous studies reported a lack of awareness of subclinical mastitis in Ethiopia (Mungube et al., 2005; Almaw et al., 2008). In our study as well farmers were not at all familiar with the concept of mastitis in the lack of visible signs. Cows were therefore never screened for the

presence of an elevated somatic cell count nor sampled for bacteriological culture to detect subclinical mastitis. Veterinary assistance was only requested in case of severe clinical mastitis. Yet, milk production losses and costs associated with subclinical mastitis in Ethiopian crossbred dairy have been estimated to be high (Mungube et al., 2005; Tesfaye et al., 2010). Therefore, informing farmers and creating awareness around subclinical mastitis in Jimma remain major goals. In this study a first attempt was undertaken using a cheap cow-side test, although the authors acknowledge culturing of milk samples would have generated more detailed insights.

The prevalence of subclinical mastitis recorded in this study was 61.9% at cow and 51.3% at quarter level as measured by CMT. A comparable study conducted using CMT in similar smallholder dairy farms around Addis Ababa reported a lower prevalence: subclinical mastitis was detected in 46.6% and 27.8% of the investigated cows and quarters, respectively (Mungube et al., 2004). However, the authors of the latter study declared quarters to be subclinically infected when the CMT score was ≥1 which explains part of the difference. In other regions of Ethiopia, lower proportions of CMT positive quarters were observed as well. Almaw et al. (2008), using the same CMT threshold to declare a quarter to be subclinically infected as in the present study, reported a prevalence of 17.9% in crossbreds in Bahir Dar while Getahun et al. (2008), using the same definition as Mungube et al. (2004), found subclinical mastitis in only 10.1 % of the tested quarters of crossbreds in Selale. In both study areas, however, cows were grazing and supplemented with concentrate whereas in Jimma, the large majority of dairy farmers apply zero-grazing, restricting the animals to stay in confined areas which eventually causes a higher infection pressure and an associated increased infection risk. Also, the central part of the country and mainly Selale is the main source for stock replacement in Jimma. Most likely, less productive animals, and eventually subclinically infected, are transported to Jimma and sold. Those differences in management

practices and stock replacement could explain the higher prevalence of subclinical mastitis in Jimma although this should be substantiated further. As mentioned before, research using bacteriological culturing of milk rather than using the CMT should be performed in Jimma to substantiate the current findings, to be able to describe the prevalence of intramammary infection and the distribution of associated pathogens, and to identify pathogen (group) specific risk factors.

When including a high number of potential risk factors, as in the present study, the probability of finding associations by chance increases substantially (Dohoo et al, 1997). Still, although twelve herd characteristics were recorded in this study, the two risk factors that remained significant in the final multilevel, multivariable logistic regression model were lactation status and tick infestation found to increase the likelihood of subclinical infection. Quarters of cows with a tick-infested udder were more likely to be subclinically infected than quarters of cows without ticks on the udder independently from the milking technique (stripping vs squeezing). Still, the effect of the milking technique on the likelihood of subclinical mastitis was modified by the presence/absence of ticks on the udder. Stripping is done by firmly holding the teat between the thumb, index and middle finger and then drawing it down the length of the teat which is very stressful for the teat. On the other hand, five finger squeezing removes milk much quicker than stripping, exerts a more equal pressure on the teat and is more similar to the natural suckling process by calf. Two Tanzanian research groups have studied the association between subclinical mastitis and milking technique before. Karimuribo et al. (2008) detected significantly less CMT positive quarters in farms practising stripping compared to farms practising squeezing in the Tanga region which is in contrast with our finding. In a study by Kivaria et al. (2004), a comparable number of cows was sampled compared to our study (n = 182) and found no association between milking technique and CMT positive quarters.

The direct effect of tick infestation to livestock has been discussed before (Jongejan and Uilenberg, 2009): ticks cause vast damage to skin, udder, and teats and often secondary bacterial infection occurs. Resistance to tick infestation differs from breed to breed; *Bos taurus* is more susceptible than *Bos indicus* (Jongejan and Uilenberg, 2004). A study from Zimbabwe revealed that udder and teat damage was significantly associated with tick infestation (Ndhlovu et al., 2009). In Ethiopia, Biffa et al. (2005) found a significant association between tick infestation and subclinical mastitis. A similar trend was seen in our data (tick infestation increases the likelihood of infection). Therefore, tick control should be included in any mastitis control program implemented in Jimma and other parts of Ethiopia, also because it relates to other diseases than mastitis only.

Of the eight cow characteristics studied, one was associated with subclinical mastitis. Similar to other Ethiopian (Mungube et al., 2004; Getahun et al., 2008; Almaw et al., 2008), and Indian (Sudhan et al., 2005) studies, quarters from cows in later lactation were more likely to be subclinically infected compared to quarters from cows in early lactation. In Jimma, cows are often milked for a very long period; actually, the average DIM of the cows included in the present study was 198 ranging between 2 to 1080. Among other reasons, the lack of artificial insemination services and fertility management causes elongation of the lactation periods. Appropriate follow-up of fertility would allow cows to be dried-off at a more appropriate moment.

A minority of the variation in the outcome variable resided at the herd level, with the majority being present at the cow and quarter levels. This is comparable to what we have described when studying pathogen group specific risk factors for intramammary infections in Belgian heifers managed under totally different conditions (Piepers et al., 2011). This suggests that udder health management between the herds did not differ substantially, whereas cows under the same management, and quarters within the same cow differ more in either

their degree of exposure to causative pathogens or their susceptibility to infection. Future research should therefore focus more on cow and quarter level factors than on herd level factors when studying udder health, also in Jimma, Ethiopia.

5. Conclusions

A high proportion of quarters and cows are subclinically infected as detected using CMT in smallholder dairy farms in Jimma, Ethiopia. A number of the studied herd and cow level characteristics were associated with subclinical mastitis as measured by CMT, but none of the quarter level factors were. Milking by squeezing instead of stripping in particular in cows without udder tick infestations, application of appropriate tick control measurements (tick-infested udders) as well as the implementation of a more appropriate fertility management, and allowing cows to be dried-off at a more appropriate moment, could reduce subclinical mastitis prevalence (and incidence) on the short term. On the long term, awareness around the presence and importance of subclinical mastitis in Jimma should be increased by studying the subject in more detail (e.g. by testing whether dry cow treatment using long-acting antibiotic preparations could have added value without jeopardizing food safety) as well as by implementing a mastitis prevention program adapted to the local needs through setting up education programs for and communication towards dairy farmers.

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Conflict of interest statement

No conflict of interest exists for any of the authors.

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Chapter 4.2:

Pathogen group specific risk factors for clinical mastitis, intramammary infection and blind quarters at the herd, cow and quarter level in smallholder dairy farms in Jimma, Ethiopia

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ABSTRACT

A cross-sectional study on clinical mastitis, intramammary infection (IMI) and blind quarters was conducted on 50 smallholder dairy farms in Jimma, Ethiopia. A questionnaire was performed, and quarters of 211 cows were sampled and bacteriologically cultured. Risk factors at the herd, cow, and quarter level for clinical mastitis and (pathogen-specific) intramammary infection were studied using multilevel modeling. As well, factors associated with quarters being blind were studied. Eleven percent of the cows and 4% of the quarters had clinical mastitis whereas 85% of the cows and 51% of the quarters were infected. Eighteen percent of the cows had one or more blind quarter(s), whereas 6% of the quarters was blind. Non-*aureus* staphylococci were the most frequently isolated pathogens in both clinical mastitis cases and IMI. The odds of clinical mastitis was lower in herds where heifers were purchased in the last year [odds ratio (OR) with 95% confidence interval: 0.11 (0.01-0.90)], old cows (>4 y) [OR: 0.45 (0.18-1.14)] and quarters not showing teat injury [OR: 0.23 (0.07-0.77)]. The odds of IMI caused by any pathogen was higher in herds not practicing teat drying before milking (opposed to drying teats with 1 towel per cow) [OR: 1.68 (1.05-2.69)], late lactation cows (> 180 DIM opposed to ≤90 DIM) [OR: 1.81 (1.14-2.88)], cows with high body condition score (BCS) (>3) [OR: 1.57 (1.06-2.31)], right quarters (opposed to left quarter position) [OR: 1.47 (1.10-1.98)] and quarters showing teat injury [OR: 2.30 (0.97-5.43)]. Quarters of cows in herds with bucket-fed calf feeding (opposed to suckling) had higher odds of IMI caused by *Staphylococcus aureus* [OR: 6.05 (1.31-27.90)]. Except for BCS, IMI caused by non-*aureus* staphylococci was associated with the same risk factors as IMI caused by any pathogen. No access to feed and water immediately after milking [OR: 2.41 (1.26-4.60)], higher parity [OR: 3.60 (1.20-10.82)] and tick infestation [OR: 2.42 (1.02-5.71)] were risk factors for quarters being blind. In conclusion, replacement of old cows, prevention of teat injuries/lesions, drying teats with 1 towel per cow before milking,

improving fertility in order to shorten the lactation period, allowing (restricted) suckling, access to feed and water immediately after milking, and improving tick control could improve udder health in Jimma.

Key words: Clinical mastitis, Intramammary infection, Prevalence, Risk factor

1. Introduction

Population growth and urbanization cause an increase in the demand for milk in cities of developing countries (Narrod et al., 2011). In response, smallholder dairy farms were established in Jimma and other Ethiopian towns to fulfill the increasing demand for dairy products (Mekonnen et al., 2006; Tolosa et al., 2013). Zebu cattle are crossbred with exotic dairy breeds in these urban dairy farms. Crossbreeds have a higher genetic merit for milk production but seem to be more susceptible to mastitis (Almaw et al., 2008).

Recently, we reported a high prevalence of subclinical mastitis in crossbreeds in Jimma, Ethiopia using the California Mastitis Test (CMT). Sixty-two percent of the cows and 51% of the quarters were diagnosed to be subclinically infected (Tolosa et al., 2013) and 2 risk factors for subclinical mastitis were identified. Overall, quarters from cows in later stage of lactation (>180 DIM) [opposed to early lactation (<90 DIM)] and quarters from cows with a tick-infested udder had higher odds of subclinical mastitis, as reflected by the CMT test. Quarters of cows without a tick-infested udder milked by squeezing were less likely to be subclinically infected than when milked by stripping. However, the milking technique did not influence the odds of infection in cows with ticks on the udder.

Using CMT, the prevalence of subclinical mastitis in smallholder dairy farms in Jimma was shown to be high (Tolosa et al., 2013) but little is known on the causative pathogens, and of the prevalence of clinical mastitis. Hence, the first objective of this study was to determine the prevalence of both clinical mastitis and IMI in Jimma through a cross-sectional study, and to identify the associated pathogens. Second, to identify potential control measures, risk factors at the herd, cow, and quarter level for clinical mastitis and IMI were screened. Because of their high prevalence, pathogen-group specific risk factors for IMI caused by *Staphylococcus aureus* (*S. aurous)* and non-*aureus* staphylococci were studied in detail. As

mastitis can lead to loss of quarter (Wenz et al., 2004) and many cows in Jimma have one or more blind (non-functional) quarter(s) (Tolosa et al., 2013), factors associated with quarters being blind were also analyzed.

2. Materials and methods

2.1. Description of the study area

Jimma town is located in Oromiya Regional State, 352 km South-West of Ethiopia's capital, Addis Ababa. The region has a tropical climate with annual rainfall ranging between 1400 and 1900 mm. The mean maximum and minimum temperature are 25-30°C and 7-12°C, respectively (Alemu et al., 2011). The area is mainly known for its coffee production but crop and livestock production are also important.

2.2. Herds and animals

Fifty out of 66 active dairy herds in Jimma were visited once by the first author between June 2012 and March 2013. Three herds were not visited because of absence of lactating cows, 7 farmers could not be contacted and 6 farmers refused to cooperate. Herd size ranged from 2 to 62 animals, young stock included. All lactating cows (n = 211) were sampled. Cows were housed in tie stalls with limited or no access to pasture and hand-milked twice daily. The average lactation stage and parity were 160 DIM (range of 2 to 730) and 2.6 (range of 1 to 8), respectively.

2.3. Data collection

Herd, cow and quarter characteristics potentially associated with clinical mastitis, (pathogen- specific) IMI and blind quarters were recorded using a questionnaire and through observation.

Herd level information - Herd information were subdivided in 4 categories. First, general farm management characteristics including farmer experience (3 categories: ≤ 13, 13-26, > 26 years), herd size (2 categories: ≤ 10 versus > 10 animals, young stock included), calf feeding (2 categories: bucket-fed versus suckling), and grazing type (2 categories: limited access to pasture versus zero-grazing) were recorded. Secondly, milking procedures and hygiene were studied in more detail; questions on the number of milkers (3 categories: 1, 2, more than 2 milkers), washing of udder before milking (3 categories: no washing, yes teats only, yes whole udder), teat drying before milking (3 categories: no drying, yes by using 1 towel for multiple cows, yes by using 1 towel per cow), pre-stripping of foremilk before milking (yes versus no), access to feed and water immediately after milking (yes versus no), milking cows with clinical mastitis as last (yes versus no), and milking technique (stripping versus squeezing) were asked. Thirdly, information on cow comfort and hygiene was noted including subjects as stable floor type (concrete versus wood or soil), straw or saw dust bedding in use (yes versus no), changing frequency of bedding per day (4 categories: no bedding, 1 time, 2 times, 3 times), frequency of manure removal per day (4 categories: 1 time, 2 time, 3 times, variable), and presence of a calving pen (yes versus no). Finally, data on herd biosecurity and prevention was recorded; purchase of heifers or cows in the last year (yes versus no) and purchase of prepartum heifers in the last year (yes versus no) were noted.

Cow level information - Age in years (2 categories: ≤ 4 versus > 4), parity (2 categories: multiparous versus primiparous), and lactation stage (3 categories: ≤ 90 DIM, 90-180 DIM, > 180 DIM) was recorded for every cow. Body condition score (5 scores: 1-5 scores) of all

sampled cows was measured as described by Edmonson et al. (1989) and categorized as low (≤3) or high BCS (>3). Udder and leg hygiene was separately scored (4 categories) as described by Schreiner and Ruegg (2002). Flank hygiene was scored using the same definitions. Cows were clinically examined for presence of tick infestation on the udder (one tick was also seen as "yes" versus not seen as "no").

Quarter level information - For each lactating quarter, position (2 x 2 categories: right versus left and hind versus front) and presence of teat injuries/lesions (2 categories: yes versus no) was noted.

2.4. Milk sample collection

Quarter milk samples were collected aseptically from all lactating cows following National Mastitis Council guidelines (NMC, 1999). Quarters were palpated and first streams of milk were inspected to detect abnormalities (see further). After collection, milk samples were kept in a cool box during transportation to the laboratory.

2.5. *Bacteriological culture*

Bacteriological culture was performed according to NMC guidelines (1999). From each sample, 10 µL of milk was plated on Colombia blood (5% sheep blood) and MacConkey agar (Oxoid, Hampshire, UK) and incubated aerobically for 24 h or 48 h at 37°C. A mammary quarter was considered culture-positive when the growth of at least one colony was detected on the streaks (≥ 100 cfu/mL). Samples yielding 2 different bacterial species were grouped as “mixed culture” whereas samples yielding more than 2 different bacterial species were considered to be contaminated and removed from the statistical analysis (n = 7, 0.8%).

Bacteria were identified based on colony morphology, Gram-staining, and conventional biochemical tests. For Gram-positive cocci, catalase tests with hydrogen peroxide (3%) were used to differentiate between catalase-positive staphylococci and catalase-negative cocci. Morphology, haemolysis patterns, and DNase, coagulase, and polymyxin susceptibility testing were used to distinguish *S. aureus* from non-*aureus* staphylococci, referred to as *Staphylococcus* spp. throughout this paper. Catalase-negative cocci were cultured on Edward aesculin media to differentiate aesculin-positive cocci and aesculin-negative streptococci (*S. agalactiae* and *S. dysgalactiae*). Christie, Atkins, Munch-Petersen (CAMP) tests were used to distinguish *S. agalactiae* and *S. dysgalactiae*. Gram-negative bacteria were identified using colony morphology, oxidase test and lactose fermentation on MacConkey agar.

2.6. Outcome variables

Five outcome variables were studied: (1) clinical mastitis, (2) IMI by any pathogen (IMI any pathogen), (3) IMI by *S. aureus* only (*S. aureus* IMI), (4) IMI by non-*aureus* staphylococci only (*Staphylococcus* spp. IMI), and (5) blind quarters. A quarter was considered having clinical mastitis if it was swollen and/or painful and/or if the milk showed visible abnormalities (flakes, clots, watery or discolored milk) regardless of the bacteriological culture results (Lakew et al., 2009). Intramammary infection was defined as growth of at least one colony of any pathogen. *Staphylococcus aureus* IMI and *Staphylococcus* spp. IMI were defined as growth of only *S. aureus* and *Staphylococcus* spp., respectively (Table 4.2.1).

2.7. *Statistical analysis*

Associations between potential herd, cow and quarter risk factors were assessed for the 5 abovementioned variables [using logistic mixed regression models (MlwiN 2.02, Centre for Multilevel Modeling, Bristol, UK)]. In all models, herd and cow were included as random effect to correct for clustering of cows within herds and quarters within cows. For each outcome variable, a stepwise model building approach as described by De Vliegher et al. (2004) was followed and odds ratio's (OR) with 95% confidence intervals (95% CI) were calculated. Model parameters were estimated using the restricted iterative generalized least squares and first order marginal quasi-likelihood method.

In a first step, univariable models were built to test univariable associations between the outcome variables and all independent variables. Statistical significance in this step was assessed at $P<0.15$. Secondly, Spearman Rank correlations between the selected significant independent variables were analyzed using SPSS software version 21 (Chicago, IL, USA) to check for multicollinearity. If 2 factors had a correlation coefficient > |0.6|, only one was further included in the multivariable analysis. In this case, "heifers or cows purchased in the last year" was found to be highly correlated with "heifers purchased in the last year" (r = 0.72) and not included further in multivariable models with clinical mastitis as outcome variable. Additionally, "limited access to grazing" and "age" were found to be highly correlated with "tick infestation" and "parity", respectively (r = 0.87 and 0.76), and not included further in the multivariable model with blind quarter as outcome variable. In a third step, the remaining independent variables were fit as fixed effects in multivariable models. Statistical significance in this step was assessed at $P < 0.10$. Non-significant variables were removed using backwards stepwise elimination and confounding was checked for: a variable was considered to act as a confounder if its removal made the regression coefficients of the

remaining variables undergo a relative change > 25% or in case the regression coefficient ranged between −0.4 and 0.4, if an absolute change > 0.1 was observed. Using these definitions, no confounders were detected. Finally, all two-way interactions between the remaining risk factors in the multivariable model were calculated and tested at $P< 0.10$.

The fit of the multivariable models was evaluated by performing the Pearson goodness-of-fit test on the fixed effects models and tested at $P< 0.05$. The statistic was not significant for clinical mastitis ($P = 0.69$), IMI any pathogen ($P = 0.27$), *Staphylococcus* spp. IMI ($P = 0.08$), and blind quarter ($P = 0.36$). The fit of the multivariable model for *S. aureus* IMI only was not tested because only one independent variable was included (see further). To check for bias, the multivariable models were run a second time using the Markov chain Monte Carlo estimation method with a burn-in length of 500 iterations and a monitoring chain length of 5,000 iterations. Results were concordant with the models using the restricted iterative generalized least squares and first order marginal quasi-likelihood estimation method. All fixed effects were significant at $P< 0.10$.

3. Results

3.1. Descriptive statistics

Table 4.2.1 shows the herd, cow and quarter prevalence of clinical mastitis, IMI any pathogen, *S. aureus* IMI, *Staphylococcus* spp. IMI and blind quarters. *Staphylococcus* spp. were the most frequently isolated pathogens (n = 27) in the quarters with clinical mastitis, followed by aesculin-positive cocci (n = 3), *S. aureus* (n = 2) and *Klebsiella* spp. (n = 2). *Staphylococcus* spp. were the most frequently isolated pathogens (n = 306) in the quarters with IMI, followed by *S. aureus* (n = 39), aesculin-positive cocci (n = 28), *S. agalactiae* (n =

15), *S. dysgalactiae* (n = 14), *S. uberis* (n = 8), *Klebsiella* spp. (n = 8), *E. coli* (n = 5) and *Pseudomonas aeruginosa (P. aeruginosa)* (n = 3). Mixed cultures were isolated from 6 quarters without clinical symptoms.

Table 4.2.1. Outcome variables, their definitions and herd, cow and quarter prevalence

Outcome variable	Abbreviation	Levels	Definition	n herds (%)[a]	n cows (%)[a]	n quarters (%)
Clinical mastitis	/	1	Clinical symptoms regardless of culture status	17 (34)	24 (11)	37 (4)
		0	No clinical symptoms regardless of culture status	23 (46)	152 (72)	756 (90)
		Missing value	Not producing milk	10 (20)	35 (17)	51 (6)
IMI by any pathogen	IMI any pathogen	1	Culture positive regardless of clinical symptoms	50 (100)	179 (85)	432 (51)
		0	Culture negative regardless of clinical symptoms	0 (0)	20 (9)	334 (40)
		Missing value	Not producing milk, under treatment or contaminated[b]	0 (0)	12 (6)	78 (9)
IMI by *Staphylococcus .aureus* only	*S. aureus* IMI	1	Culture positive for *S. aureus* regardless of clinical symptoms	22 (44)	28 (13)	41 (5)
		0	Culture negative regardless of clinical symptoms	0 (0)	20 (9)	334 (40)
		Missing value	Not producing milk, under treatment, contaminated and/or culture positive for other pathogens than *S. aureus*	28 (56)	163 (77)	469 (56)
IMI by non-*aureus* staphylococci only	*Staphylococcus* spp. IMI	1	Culture positive for non-*aureus* staphylococci regardless of clinical symptoms	48 (96)	160 (76)	308 (36)
		0	Culture negative regardless of clinical symptoms	0 (0)	20 (9)	334 (40)
		Missing value	Not producing milk, under treatment, contaminated and/or culture positive for other pathogens than non-*aureus* staphylococci	2 (4)	31 (15)	202 (24)
Quarter being blind	Blind quarter	1	Not producing milk	18 (36)	39 (18)	51 (6)
		0	Producing milk regardless of clinical symptoms and culture status	32 (64)	172 (82)	793 (94)

[a]Herds and cows with one positive (level 1) quarter were categorized as positive. Herds and cows without positive quarters or quarters with missing values were categorized as negative (level 0).

[b]Samples yielding more than 2 different bacterial species were considered to be contaminated and removed from the statistical analysis (n = 7)

Table 4.2.2. Final multilevel, multivariable logistic regression model describing the association between herd, cow and quarter level risk factors and clinical mastitis in 50 smallholder dairy herds in Jimma town, Ethiopia.

Independent variable	n[a]	p[b]	β[c]	SE[d]	*P*-value[e]
Intercept			-2.24	0.41	
Variance at the herd level			0.11	0.44	
Variance at the cow level			2.87	1.01	
Heifers purchased in the last year (herd)					0.04
No	42	0.06	Ref.[f]		
Yes	8	0.01	-2.23	1.08	
Age (cow)					0.09
≤4 years	49	0.08	Ref.		
>4 years	162	0.03	-0.80	0.48	
Teat injury (quarter)					0.02
No	810	0.04	Ref.		
Yes	34	0.24	1.48	0.62	

[a] Number of herds, cows or quarters.

[b] Proportion positive quarters.

[c] Regression coefficient.

[d] Standard error of the mean.

[e] *P*-value for overall effect.

[f] Reference.

3.2. Risk factors

The odds of clinical mastitis was lower in herds where heifers were purchased in the last year [odds ratio (OR) with 95% confidence interval: 0.11 (0.01-0.90)], old cows (>4 y) [OR: 0.45 (0.18-1.14)] and quarters not showing teat injury [OR: 0.23 (0.07-0.77)] (Table 4.2.2).

The odds of IMI any pathogen was associated with teat drying before milking, lactation stage, BCS, quarter position (right versus left) and teat injury (Table 4.2.3). Quarters from cows on herds practicing teat drying before milking with 1 towel per cow had lower odds opposed to herds not practicing teat drying [OR: 0.59 (0.37-0.96)] whereas practicing teat drying before milking with 1 towel for multiple cows had little influence on the odds [OR: 1.27 (0.76-2.12)]. High BCS (> 3 opposed to ≤ 3) was associated with higher odds of IMI any pathogen [OR: 1.57 (1.06-2.31)]. Quarters from cows 90-180 DIM and cows > 180 DIM had

higher odds [OR: 1.34 (0.84-2.14) and 1.81 (1.14-2.88), respectively] compared to cows ≤ 90 DIM. Right quarters and quarters with teat injury had higher odds of IMI any pathogen compared to left quarters and quarters without teat injury, respectively [OR: 1.47 (1.10-1.98) and 2.30 (0.97-5.43), respectively].

Table 4.2.3. Final multilevel, multivariable logistic regression model describing the association between herd, cow and quarter level risk factors and intramammary infection caused by any pathogen in 50 smallholder dairy herds in Jimma town, Ethiopia

Independent variable	n[a]	p[b]	β[c]	SE[d]	*P*-value[e]
Intercept			-0.35	0.22	
Variance at the herd level			0.04	0.09	
Variance at the cow level			0.71	0.20	
Teat drying before milking (herd)					0.03
No drying	27	0.58	Ref.[f]		
Yes, 1 towel for multiple cows	9	0.64	0.24	0.26	
Yes, 1 towel per cow	14	0.47	-0.52	0.24	
Lactation stage (cow)					0.05
≤ 90 DIM[g]	77	0.49	Ref.		
90-180 DIM	67	0.57	0.29	0.24	
> 180 DIM	67	0.64	0.59	0.24	
Body condition score[h] (cow)					0.02
Score 1-3	121	0.53	Ref.		
Score 4-5	90	0.61	0.45	0.20	
Quarter position (quarter)					0.01
Left	422	0.52	Ref.		
Right	422	0.61	0.39	0.15	
Teat injury (quarter)					0.06
No	810	0.56	Ref.		
Yes	34	0.70	0.83	0.44	

[a] Number of herds, cows or quarters.

[b] Proportion positive quarters.

[c] Regression coefficient.

[d] Standard error of the mean.

[e] *P*-value for overall effect.

[f] Reference.

[g] Days in milk.

[h] As in Edmonson et al. (1989).

Quarters of cows in herds with bucket-fed calf feeding (opposed to suckling) had higher odds of *S. aureus* IMI [OR: 6.05 (1.31-27.90)] (Table 4.2.4).

The odds of *Staphylococcus* spp. IMI was associated with teat drying before milking, lactation stage, quarter position (right versus left) and teat injury (Table 4.2.5). Quarters from cows on herds practicing teat drying before milking with 1 towel per cow had lower odds opposed to herds not practicing teat drying [OR: 0.60 (0.37-0.96)] whereas practicing teat drying before milking with 1 towel for multiple cows had little influence on the odds [OR: 1.21 (0.73-2.00)]. Quarters from cows 90-180 DIM and cows > 180 DIM had higher odds [OR: 1.34 (0.83-2.18) and 1.76 (1.09-2.84), respectively] compared to cows ≤ 90 DIM. Right quarters and quarters with teat injury had higher odds of *Staphylococcus* spp. IMI compared to left quarters and quarters without teat injury, respectively [OR: 1.60 (1.16-2.21) and 2.31 (0.96-5.59), respectively].

Table 4.2.4. Final multilevel, multivariable logistic regression model describing the association between herd, cow and quarter level risk factors and intramammary infection caused by *Staphylococcus aureus* in 50 smallholder dairy herds in Jimma town, Ethiopia

Independent variable	n[a]	p[b]	β[c]	SE[d]	*P*-value[e]
Intercept			-3.70	0.75	
Variance at the herd level			0		
Variance at the cow level			2.18	0.76	
Calf feeding (herd)					0.02
Suckling	14	0.03	Ref.[f]		
Bucket-fed	36	0.13	1.80	0.78	

[a] Number of herds, cows or quarters.

[b] Proportion positive quarters.

[c] Regression coefficient.

[d] Standard error of the mean.

[e] *P*-value for overall effect.

[f] Reference

Table 4.2.5. Final multilevel, multivariable logistic regression model describing the association between herd, cow and quarter level risk factors and intramammary infection caused by *Staphylococcus* spp. in 50 smallholder dairy herds in Jimma town, Ethiopia

Independent variable	n[a]	p[b]	β[c]	SE[d]	*P*-value[e]
Intercept			-0.52	0.21	
Variance at the herd level			0		
Variance at the cow level			0.64	0.20	
Teat drying before milking (herd)					0.04
No drying	27	0.50	Ref.[f]		
Yes, 1 towel for multiple cows	9	0.55	0.19	0.26	
Yes, 1 towel per cow	14	0.38	-0.52	0.24	
Lactation stage (cow)					0.07
<= 90 DIM[g]	77	0.41	Ref.		
90-180 DIM	67	0.48	0.30	0.25	
> 180 DIM	67	0.56	0.57	0.24	
Quarter position (quarter)					<0.01
Left	422	0.42	Ref.		
Right	422	0.54	0.47	0.16	
Teat injury (quarter)					0.06
No	810	0.47	Ref.		
Yes	34	0.66	0.84	0.45	

[a] Number of herds, cows or quarters.

[b] Proportion positive quarters.

[c] Regression coefficient.

[d] Standard error of the mean.

[e] *P*-value for overall effect.

[f] Reference.

[g] Days in milk.

The odds of a quarter being blind was associated with access to feed and water immediately after milking, parity and tick infestation (Table 4.2.6). Access to feed and water immediately after milking was associated with lower odds [OR: 0.41 (0.18-0.96)]. Quarters of multiparous cows (opposed to heifers) and tick infested cows (opposed to cows with udder no ticks infested) had higher odds of being blind [OR: 3.60 (1.20-10.82) and 2.42 (1.02-5.71), respectively].

Interactions were non-significant and therefore removed from the models.

Table 4.2.6. Final multilevel, multivariable logistic regression model describing the association between herd, cow and quarter level risk factors and quarters being blind in 50 smallholder dairy herds in Jimma town, Ethiopia.

Independent variable	n[a]	p[b]	β[c]	SE[d]	*P*-value[e]
Intercept			-3.51	0.65	
Variance at the herd level			0.08	0.26	
Variance at the cow level			0.76	0.58	
Access to feed and water immediately after milking (herd)					0.05
No	8	0.15	Ref.[f]		
Yes	42	0.04	-0.88	0.43	
Parity (cow)					0.02
Heifer	59	0.02	Ref.		
Multiparous cow	152	0.08	1.28	0.56	
Tick infestation (cow)					0.06
No	175	0.04	Ref.		
Yes	36	0.17	0.88	0.44	

[a] Number of herds, cows or quarters.

[b] Proportion positive quarters.

[c] Regression coefficient.

[d] Standard error of the mean.

[e] *P*-value for overall effect.

[f] Reference

4. Discussion

The objectives of this study were to estimate the prevalence and pathogen-distribution of clinical mastitis and IMI and to identify pathogen-group specific risk factors at the herd-, cow- and quarter-level in Jimma, Ethiopia. Additionally, risk factors at the herd-, cow- and quarter-level for quarters being blind were screened. The results revealed serious udder health problems confirming our previous preliminary findings (Tolosa et al., 2013). Previously, the prevalence of clinical mastitis in different regions of Ethiopia has been estimated several times (Mungube et al., 2004; Getahun et al., 2008; Lakew et al., 2009; Haftu et al., 2012). In the present study, the prevalence of clinical mastitis at the cow level was estimated to be 11% which is higher than figures reported in Selale (2%) (Getahun et al., 2008), Mekelle (4%)

(Haftu et al., 2012), and Addis Ababa (7%) (Mungube et al., 2004) but lower than the 27% in Asella reported by Lakew et al. (2009). Longitudinal studies are required to estimate the incidence rate of clinical mastitis in Ethiopia and compare it with estimates in Western countries (e.g. Olde Riekerink et al., 2008; Verbeke et al., 2014).

Unlike other Ethiopian studies (Getahun et al., 2008; Lakew et al., 2009; Haftu et al., 2012), not only CMT positive but all lactating quarters were sampled. The prevalence of IMI was 85 and 51% at cow- and quarter- level, respectively. Our estimates indicate a much higher prevalence of IMI in Jimma compared to Western regions. For example, using similar definition as in the current study, prevalence of IMI in Flanders (Belgium) was estimated at 41% at the cow and 17% at the quarter level (Piepers et al., 2007).

Although considered to be minor pathogens, *Staphylococcus* spp. were the most frequently isolated pathogens in clinical mastitis cases. In other clinical mastitis studies, *Staphylococcus* spp. was less frequently isolated (Bradley et al., 2007; Olde Riekerink et al., 2008; Keane et al., 2013; Verbeke et al., 2014). However, figures varied from less than 1% of the cases in Canadian herds (Olde Riekerink et al., 2008) to 13% of the cases in British herds (Bradley et al., 2007). In Canada and Ireland, *S. aureus* was the most frequently isolated pathogen in clinical mastitis samples (Olde Riekerink et al., 2008; Keane et al., 2013) whereas *S. uberis* was most frequently isolated in Flanders and the UK (Bradley et al., 2007; Verbeke et al., 2014). Further investigation is suggested to elucidate the different reportings.

Purchase of heifers enables producers to replace chronically infected cows and was associated with lower odds of clinical mastitis. However, quarters of older cows had lower odds of clinical mastitis compared to quarters of younger cows which is in contrast with research in developed countries demonstrating a higher incidence rate of clinical mastitis in multiparous cows compared to heifers (Barkema et al., 1998; McDougall et al., 2007; Verbeke et al., 2014). Yet, quarters of multiparous cows had higher odds of being blind compared to

quarters of heifers. Similar to Bhutto et al. (2010) and Biffa et al. (2005), presence of teat injuries or lesions was found to be a risk factor for clinical mastitis, IMI any pathogen and *Staphylococcus* spp. IMI. The teat skin forms the first line of defense against mastitis pathogens and loses its protective characteristics when injured, facilitating bacterial colonization (Paduch et al., 2012). Similar to Tanzanian research (Kivaria et al., 2004), using a single towel for teat drying of each cow before milking was associated with lower odds of IMI any pathogen and *Staphylococcus* spp. IMI. Using a single towel for each cow prevents transmission of mastitis pathogens (Kivaria et al., 2004) and should be advised to all producers. In agreement with other Ethiopian studies (Mungube et al., 2004; Almaw et al., 2008; Getahun et al., 2008; Tolosa et al., 2013), quarters from cows in later lactation had higher odds of IMI any pathogen and *Staphylococcus* spp. IMI compared with quarters from cows in early lactation. This finding is probably related to the inadequate milking routine on many farms in Jimma, provoking a fast cow-to-cow transmission of bacteria. An increase in the rate of new *S. aureus* IMI over lactation was also recently observed by Schukken et al. (2014). Lack of artificial insemination services and fertility management causes elongation of the lactation periods in Jimma (Tolosa et al., 2013). Consequently, cows benefit less from dry periods in which spontaneous cure often occurs (Halasa et al., 2009). Although several studies identified low BCS as a risk factor for mastitis (Mungube et al., 2004; Sarker et al., 2013), quarters from cows with high BCS had higher odds of IMI any pathogen compared to quarters from cows with low BCS. However, the association with BCS has no clear explanation for targeting the risk factor for prevention. Right quarters had higher odds of IMI any pathogen and *Staphylococcus* spp. IMI compared to left quarters. The right side position of the farmers during milking might explain the latter association as teats on the right side make contact with arms and hands of the milkers. Washing hands and arms with soap before milking could prevent new IMI. Additionally, lying behavior could explain the higher odds of infection in

right quarters (Ewbank, 1966) although this was not observed in loose house systems in more recent studies (Forsberg et al., 2008).

Bucket calf feeding was associated with higher odds of *S. aureus* IMI which is in accordance with an experimental study in Mexico demonstrating a positive effect on udder health of restricted suckling compared to artificial rearing (Froberg et al., 2007). In latter study, artificially reared calves displayed more cross-suckling, a known risk factor for heifer mastitis (De Vliegher et al., 2012). In herds in Jimma practicing artificial rearing, calves are refrained from suckling but often housed in the same stable as adult cows.

Quarters of tick infested cows had higher odds of being blind. Tick infestation causes damage to skin, udder, and teats, facilitates bacterial infection and was found to be associated with subclinical mastitis before (Jongejan and Uilenberg, 2004; Biffa et al., 2005; Tolosa et al., 2013). As ticks were only observed in herds with limited access to pasture, practicing zero-grazing could reduce the prevalence of tick infestation.

5. Conclusions

The prevalence of clinical mastitis and IMI in smallholder dairy farms in Jimma was estimated at 4 and 51% at quarter level and 11 and 85% at cow level through a large cross-sectional study. A relative large proportion of cows had one or more blind quarter(s). Non-*aureus* staphylococci were the most frequently isolated pathogens in both quarters having clinical mastitis and infected quarters. A number of the studied herd, cow, and quarter characteristics were associated with clinical mastitis, (pathogen-group specific) IMI or quarters being blind. Replacement of chronically infected cows and prevention of teat lesions could reduce clinical mastitis prevalence. Drying teats with 1 towel per cow before milking and improving fertility, allowing cows to be dried-off at a more appropriate moment, could

reduce overall and *Staphylococcus* spp. IMI prevalence. Allowing suckling could reduce *S aureus* IMI. Providing feed and water immediately after milking and improving tick control could prevent loss of quarters.

Conflict of interest statement

No conflict of interest exists for any of the authors.

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CHAPTER 5:

General discussion

1. Introduction

Agriculture and food quality awareness differ largely between developed and developing countries (Kearney, 2010). In this thesis, milk quality and udder health were studied in Jimma, Ethiopia, as well as milk production and marketing chains. The results will be useful to improve the milk quality and udder health in that region. Moreover, the findings are valuable in formulating guidelines for Ethiopia and neighboring countries.

2. Dairy industry in Jimma

2.1. Raw milk consumption

Raw milk consumption forms a risk for food borne pathogens and toxins (Wilcock et al., 2004; Oliver et al., 2005; Petrovski et al., 2006). In Jimma, the majority of the adults and even some children were found to drink unpasteurized milk (**Chapter 3.1)**. Consumption of raw milk can be a risk for zoonotic diseases caused by pathogens such as *B. abortus*, and *M. bovis*. Contact with oxacillin-resistant strains of *S. aureus,* abundantly present in the milk of Jimma (**Chapter 3.1**), further threatens public health (Haran et al., 2012). This suggests that a major problem exists in Jimma and the whole of Ethiopia needing attention of concerned bodies (e.g. government, dairy industry, farmers, and consumers). Raising awareness to boil raw milk before consumption can reduce food borne infections. Respecting withdrawal period and discarding milk from treated cows can also minimize risk of infection by resistant bacteria.

2.2. Milk chain

Similar to other Ethiopian regions (Yilma et al., 2011) and India (Hemme et al., 2001), the milk flow in Jimma was found to be poor (**Chapter 3.1**). Unlike most developed countries (Ruegg and Pantoja, 2013), milk was directly sold from the dairy farm to retailers and consumers without quality control. Yet, since recently a limited proportion of milk passes through milk collection centres in Jimma, which opens up perspectives for milk quality control.

As no processing plants were present in Jimma, we studied the milk flow in a convenience sample of 8 milk processing plants in and around Addis Ababa, the capital of Ethiopia (data unpublished). Eight out of 12 contacted plants showed the willingness to collaborate for an interview. We did not think of pre-testing the questionnaires used in these studies. Pre-testing questions is used to identify problems that might lead to biased answers. The milk flow in and around Addis Ababa was found to be direct from cooperatives or farms to the milk processing plants. Otherwise, just as in Jimma and elsewhere in Ethiopia, the milk flow goes directly from producers to consumers or retailers without any legal milk control (**Chapter 3.1**., Gonfa et al., 2001; Yilma et al., 2011; Makita et al., 2012).

The milk flow in Jimma is constrained by a low demand of milk during fasting period, shortage of animal feed and a low milk price (**Chapter 3.1**, see Table 5.1) which concordance with previous reports (Negash et al., 2012). Part of a solution could be that fresh, high quality milk is locally processed to heat-treated milk, cheese, butter, and milk powder with a longer shelf life. The local cooperative should be more proactive in that area, with the support of the government or non-governmental organisations (e.g., Ministry of Livestock and Fishery Resources, United States Agency for International Development, Food and Agriculture Organization of the United Nations, The Netherlands Development Organisation, Bill &

Melinda Gates Foundation). Even at the farm level, processing of excess milk could be a (partial) solution. All in all this approach could be a driver for improving milk quality as well.

Table 5.1. Comparison of different studies on the main constraint for the milk flow in different regions of Ethiopia

Constraints	Debre Zeit[1]	Mid-Rift Valley [2]	Bahir Dar[3]	Jimma[4]	References
Low demand for milk during fasting		■		■	Negash et al., 2012, Current PhD study (Chapter 3.1)
Shortage of animal nutrition	■		■	■	Mekonnen et al., 2006, Roschinsky et al., 2015, Current PhD study (Chapter 3.1)
Low milk price		■		■	Negash et al., 2012, Current PhD study (Chapter 3.1), Roschinsky et al., 2015
Cattle health/reproduction service	■		■	■	Mekonnen et al., 2006 Current PhD study (Chapter 3.1), Roschinsky et al., 2015
Increased workload/management			■		Roschinsky et al., 2015
Unsatisfactory support services			■	■	Current PhD study (Chapter 3.1); Roschinsky et al., 2015
Input shortage	■		■		Mekonnen et al., 2006, Roschinsky et al., 2015
Marketing/financial problems	■	■	■		Negash et al., 2012, Roschinsky et al., 2015

[1]Central Oromia,
[2]Amhara region
[3]Western Oromia

3. Milk quality and safety

This thesis demonstrated milk quality control in Jimma to be poor (**Chapter 3.1**) except for a small proportion milk passing through the milk collection centers. Similarly, the milk quality control in and around Addis Ababa was limited to an alcohol and a specific gravity test at processing plants, cooperatives or milk collection centers (data unpublished; Table 5.2). To say the least, the results from the questionnaire are striking.

Table 5.2. Information on milk quality control on 8 interviewed milk processing plants in and around Addis Ababa

Description	Number	Positive response (%)
Do you have knowledge of legal requirements on milk processing?	8	2 (25)
Do you have knowledge of legal requirements on transport of milk from farm to processing plant?	8	2 (25)
Do you have knowledge of legal requirements on milk dispatching ?	8	1 (12.5)
Do you perform an organoleptic test?	8	2 (25)
Do you perform clot on boiling test?	8	2 (25)
Do you perform an alcohol test?	8	2 (25)
Do you perform an acidity test?	8	2 (25)
Do you use a lactometer test?	8	2 (25)
Do you test for the presence of antimicrobial residues?	8	1 (12.5)

Overall, we conclude the absence of a milk quality testing, premium or penalty system in Jimma to be one of the reasons for poor quality milk production. Still, premium programs typically encourage farmers towards the production of better quality milk (Reneau, 2001; Botaro et al., 2013). As compared to EU and US, implementation of a premium payment system in Ethiopia is more economically feasible because of three main reasons: 1. presence of cheap labor for working on a farm, 2. high milk price and 3. consumers' willingness to pay more for proven milk quality. Hence, both producers and processing plants could benefit from a premium payment system. Adulteration is the main constraint for the milk retailers (**Chapter 3.1**). Stringent control by government and penalties for processing plants not complying with the existing regulations would curb the milk quality problem in the study area at least partially (Fig 5.1).

The high TBC and CC in bulk milk in Jimma (**Chapter 3.1**) suggests the presence of unhygienic milking and processing in the farms comparable with reports from Tanzania (Kivaria et al., 2006). From a more practical point of view, implementation of proper cleaning and disinfection of the milk containers and cooling throughout the milk chain could improve the milk quality (Bonfoh et al. 2006). We also found high SCC values in bulk milk samples (**Chapter 3.1,** see Table 5.3) comparable with Tanzania report (79%) (Kivaria et al., 2006).

SCC increases in milk when a higher proportion of cows have mastitis whereas a lower value indicates good udder health (Ruegg and Pantoja, 2013). Unlike the developed world, Ethiopia has no legal standard for the SCC of raw milk. The SCC, TBC, and CC reported are much higher than international acceptable limits for raw milk (**Chapter 3.1**) suggesting training, coaching, better communication, implementation of penalty and premium programs for farmers, cooperatives, and processing plants will be useful to improve the quality milk supply in Jimma (Figure 5.1).

A higher proportion of bulk milk samples contained antimicrobial residues (Table 5.2). The questionnaire in Addis Ababa demonstrated a lack of knowledge on antimicrobial residues screening. This indicates interviewees have either poor knowledge on legal requirements for milk quality control and/or a residue testing is overlooked or even neglected deliberately (data unpublished; Table 5.1; Table 5.2). The Ethiopia standards describes milk is unfit for human consumption if a minimum concentration of antimicrobials is detectable in the raw milk. (QSAE, 2009). Nevertheless, neither milk quality testing nor penalty systems are applied in Ethiopia. Antimicrobial residues enter human food system when treated cows' milk is not discarded. At the farm level, veterinarians should diagnose, supervise, coach, prepare guidelines and advise on appropriate treatments. Marking and isolation of cows with mastitis when treated, respecting withdrawal periods, and discarding milk would eventually reduce the risk of antimicrobial residues entering the human food chain.

Table 5.2. Milk quality parameters of smallholder dairy farms (n=47) in Jimma compared with other countries

Country	Parameter				Reference
	BMSCC	TBC	CC	Residues	
Jimma	609,500 cells/mL	122,500 cfu/mL	1,005 cfu/mL	20%	Current PhD study (Chapter 3.1)
Tanzania	79.0% positive to CMT	>6,000,000 cfu/mL	NI[a]	7%	Kivaria et al. (2006)
USA	206,400 cell/mL	12,500 CFU/mL	242 CFU/mL	NI[a]	Pantoja et al. (2009)

[a]Information is not available

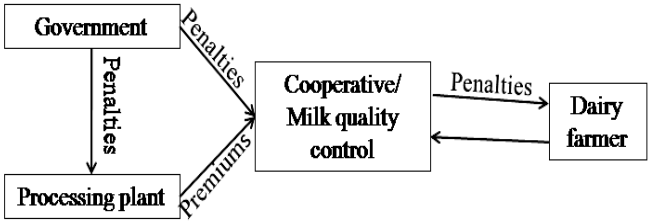

Figure 5.1. Implementation of a premium program or penalty program for quality milk production.

4. Mastitis

4.1. Prevalence

In **Chapter 4.1** and **Chapter 4.2,** we described a high proportion of subclinical mastitis and IMI cases in quarters and cows by using CMT and bacteriological culture, respectively. Sampling was performed by the PhD candidate. Overall, we conclude mastitis to be an important issue in Ethiopia as no Ethiopian study reports a low prevalence of both clinical and subclinical mastitis (Kerro Dego and Tarek, 2002 Almaw et al., 2008; Getahun et al., 2008;

Haftu et al., 2012). In our study, all lactating quarters were sampled and cultured which was not done by other Ethiopian researchers before (Getahun et al., 2008; Lakew et al., 2009; Haftu et al., 2012) although the incidence and average duration of clinical mastitis cases in Jimma remain to be estimated in a well-designed cohort study. The prevalence of blind quarters has been reported earlier in Ethiopia (Getahun et al., 2008; Lakew et al., 2009) and the USA (Duraes et al., 1982). However, it was higher in the current study.

4.2. Pathogen distribution

Staphylococcus spp. were the most frequently isolated pathogens in the quarters with clinical mastitis and IMI (**Chapter 4.2**). In contrast, Lakew et al. (2009) reported *S. aureus* as the most dominant pathogens whereas Workineh et al. (2002) and Haftu et al. (2012) reported *E. coli* as the dominant pathogen causing clinical mastitis. Many European authors have reported that non-*aureus staphylococci* are infrequently causing clinical mastitis (typically mild cases) and consider them to be minor pathogens (Bradley, 2002; Bradley et al., 2007; Piepers et al., 2007) opposing our findings. Others reported *Staphylococcus* spp. to be the most common organisms isolated from clinical cases (Lafi et al., 1994), supporting our findings. Lack of teat dipping and blanket dry cow treatment are risk factors for *Staphylococcus* spp. in Europe and North-America (Plozza et al., 2011). Similarly, Abrahmsén et al. (2014) from Uganda reported a high prevalence of SCM and *Staphylococcus* spp. were the most common isolates identified in the smallholder dairy farms in Kampala. Study herds were kept under similar zero-grazing conditions and the sample size was comparable with our study in Jimma. We hypothesize the high prevalence of *Staphylococcus* spp. in Jimma might be due to lack of post-milking teat dipping and blanket dry cow treatment. Further research on whether *Staphylococcus* spp. in Jimma and Uganda are more

virulent than strains in Europe and North-America could be studied *in vitro* or using mastitis mouse models (Breyne et al., 2015). In a well-designed experimental study, this hypothesis can be tested by comparing the virulence of bacterial strains of the abovementioned countries with the Ethiopian strains. Presence or absence of virulence factors of each strain could than be associated with clinical symptoms, SCC, and CFU.

4.3. Risk factors

Identification of risk factors is substantial for the design of control strategies for diseases such as mastitis. Previous studies in Ethiopia have reported on herd- and cow-level risk factors (Mungube et al., 2004; Almaw et al., 2008; Getahun et al., 2008; Tolosa et al., 2013; see Table 5.4 and Table 5.5).

Yet only some factors increased the odds of mastitis in our work such as not purchasing heifers in the last year, lack of access to feed and water immediately after milking, and high body condition score, were also identified. As some practices were not applied on any of the farms (e.g. teat dipping and disinfection), they could not be studied.

Combining all of these information **(**Table 5.4 and Table 5.5), we believe that an Ethiopian mastitis prevention and control program should focus on the following manageable risk factors: applying teat drying with 1 towel per cow before milking, avoiding teat lesions by adding straw as bedding, teat dipping with emollient-holding products and by improving housing in general. In addition, culling of chronically infected cows, replacing old cows, prevention of ticks by spraying acaricides on infested cows and practising zero-grazing, and shortening of lactation length by improving fertility management and allowing dry-off cows at more appropriate moment (e.g. during the dry season) would improve udder health substantially.

Table 5.4. Overview of risk factors for subclinical mastitis (CMT positive milk sample) in Ethiopia and Uganda.

Risk factors	Likelihood	Levels	Reference	Countries
↑Parity	↑	3 (1, 2, 3)	Mungube et al.(2004)	Ethiopia
↑Parity	↑	2 (Primiparous, multiparous)	Abrahmsén et al. (2014)	Ethiopia
↑Parity	↑	7 (1; 2. .....6, >6)	Kerro Dego and Tareka (2002)	Ethiopia
↑Parity	↑	4 (1, 2, 3, >3)	Lakew et al. (2009)	Ethiopia
↑Parity	↑	4 (1, 2, 3-5, >5)	Almaw et al. (2008)	Ethiopia
↑parity	↑	3 (1-3, 4-7, >7)	Haftu et al. (2012)	Ethiopia
↑Lactation stage	↑	2 (Mid, late)	Mungube et al.(2004)	Ethiopia
↑Lactation stage	↑	3 (Early, mid, late)	Getahun et al. (2008)	Ethiopia
↑Lactation stage	↑	3 (Early, mid, late)	Haftu et al. (2012)	Ethiopia
↑Lactation stage	↑	3 (< 90 DIM, >90-180 DIM, >180 DIM)	Current PhD study (Chap 4.1)	Ethiopia
↓Body score	↑	3 (Good, fair, poor)	Mungube et al.(2004)	Ethiopia
Breed	↑	2 (Holstein, Other breeds)	Abrahmsén et al. (2014)	Uganda
Breed	↑	3 (Zebu, Jersy, Holstein)	Kerro Dego and Tareka (2002)	Ethiopia
Breed	↑	2 (Cross, Zebu)	Alma et al. (2008)	Ethiopia
Breed	↑	2 (Cross, Zebu)	Lakew et al. (2009)	Ethiopia
↑Lesion or tick on teat/udder	↑	2 (Present, absent)	Alma et al. (2008)	Ethiopia
Tick infestation of udder (cow)	↑	2 (Yes, no)	Current PhD study, (Chap 4.1	Ethiopia
Lesion on teat/udder	↑	2 (Yes, no)	Kerro Dego and Tareka (2002)	Ethiopia
Lesion on teat/udder	↑	2 (Yes, no)	Getahun et al. (2008)	Ethiopia
Lesion on teat/udder	↑	2 (Present, absent)	Lakew et al. (2009)	Ethiopia
↓Hygiene milking process	↑	2 (poor, good)	Lakew et al. (2009)	Ethiopia
↓Managing dairy farming	↑	2 (main activity, sideline)	Mekonnen and Tesfaye (2010)	Ethiopia
↑Farming experience	↓	3 (≤4 y, 5-8 y, >8 y)	Mekonnen and Tesfaye (2010)	Ethiopia
↓Barn hygiene condition	↑	3 (Good, fair, poor)	Mekonnen and Tesfaye (2010)	Ethiopia
Previous mastitis history	↑	2 (Yes, no)	Mekonnen and Tesfaye (2010)	Ethiopia

Table 5.5. Overview of risk factors for mastitis (clinical mastitis, subclinical mastitis, intramammary infection) in Ethiopia, Macedonia, and Australia.

Risk factors	Clinical mastitis	Level	Reference	Countries
↑Age (cow)	↑clinical mastitis	2 (Middle, old)	Mungube et al.(2004)	Ethiopia
↑Age (cow)	↓	2 (≤4 years, >4 years)	Current PhD study (Chap 4.2)	Ethiopia
↑parity	↑	3 (1, 2, 3)	Mungube et al.(2004)	Ethiopia
↑parity	↑	6 (1, 2 , 3, 4, 5, ≥6)	Nakov et al (2014)	Macedonia
↑parity	↑	5 (1, 2, 3-6, 7-9, ≥10)	Hammer et al. (2012)	Australia
↑Lactation stage	↑	2 (Mid, late)	Mungube et al.(2004)	Ethiopia
↓Body score	↑	3 (Good, fair, poor)	Mungube et al.(2004)	Ethiopia
↑Leaking milk	↑	2 (Yes, no)	Mungube et al.(2004)	Ethiopia
Previous udder problem	↑	2 (Yes, no)	Mungube et al.(2004)	Ethiopia
↑Heifers purchased in the last year (herd)	↓	2 (Yes, no)	Current PhD study (Chap 4.2)	Ethiopia
Teat injury (quarter)	↑	2 (No, yes)	Current PhD study (Chap 4.2)	Ethiopia
Quarter position (quarter)	↑	2 (Rear quarter, Fore quarter	Nakov et al (2014)	Macedonia
Risk factors	Subclinical mastitis	Level	Reference	
↑Age (cow)	↑ Subclinical mastitis	2 (Middle, old)	Mungube et al.(2004)	Ethiopia
↑parity	↑	3 (1, 2, 3)	Mungube et al.(2004)	Ethiopia
↑Lactation stage	↑	2 (Mid, late)	Mungube et al.(2004)	Ethiopia
↑Lactation stage	↑	3 (Beginning, mid, end)	Getahun et al. (2008)	Ethiopia
↑Lactation stage	↑	3 (< 90 DIM, >90-180 DIM, >180 DIM)	Current PhD study (Chap 4.1)	Ethiopia
↓Body score	↑	3 (Good, fair, poor)	Mungube et al.(2004)	Ethiopia
Lesion on teat/udder	↑	2 (Yes, no)	Getahun et al. (2008)	Ethiopia
Tick infestation of udder (cow)	↑	2 (No, yes)	Current PhD study (Chap 4.1)	Ethiopia
Risk factors	intramammary infection	Level	Reference	Countries
Teat drying before milking (herd)	↓ by any pathogens	3 (No drying, Yes, 1 towel for multiple cows, Yes, 1 towel for one cows)	Current PhD study (Chap 4.2)	Ethiopia
↑Lactation stage	↑	3 (< 90 DIM, >90-180 DIM, >180 DIM)	Current PhD study (Chap 4.2)	Ethiopia
↑Body condition score	↑	2 (Score 1-3, Score 4-5)	Current PhD study (Chap 4.2)	Ethiopia
Quarter position (Right quarter)	↑	2 (Left, right)	Current PhD study (Chap 4.2)	Ethiopia
Teat injury (quarter)	↑	2 (No, yes)	Current PhD study (Chap 4.2)	Ethiopia

Calf feeding (herd)	↑by *Staphylococcus aureus*	2 (Suckling, bucket feeding)	Current PhD study (Chap 4.2)	Ethiopia
Teat drying before milking (herd)	↓ by *Staphylococcus* spp	3 (No drying, Yes, 1 towel for multiple cows, Yes, 1 towel for one cows)	Current PhD study (Chap 4.2)	Ethiopia
↑Lactation stage	↑	3 (< 90 DIM, >90-180 DIM, >180 DIM)	Current PhD study (Chap 4.2)	Ethiopia
Quarter position (Right quarter)	↑	2 (Left, right)	Current PhD study (Chap 4.2)	Ethiopia
Teat injury (quarter)	↑	2 (No, yes)	Current PhD study (Chap 4.2)	Ethiopia

5. Practical applications

Following suggestions are made to limit public health risks and to improve milk quality and udder health in Jimma:

- Raising awareness of consumers, retailers, milk processing plants and farmers through mass media, pamphlets, posters and training on hygienic milking techniques and milk handling from grass (farm) to glass (selling point), use of cold chains facility, and consumption raw milk after boiling are important steps for minimising cross-contamination and growth of any microorganism present in the raw milk and will improve milk quality and reduces the risk of food borne infection.
- Implementing milk quality testing reduces adulteration, bacterial contamination and antimicrobial residues. This will contribute for quality milk supply if it is supported with:
    - Provision of a temporary and renewable certificate for "high-quality milk":- reduced somatic cell count, total bacterial count coliform count, and risk of antimicrobials in raw bulk milk are the criteria needed
        - Exercising incentives program
        - Applying graded differential payment for better quality milk
        - Developing educational material on mastitis and milk quality
- Advising farmers to discard milk from treated cows and respecting withdrawal period is minimizing antimicrobial residues risks both for consumer health, and the processing plants. The role of the veterinarian in this regard is high in training farmers and prescribing guidelines on the proper use of antimicrobials.
- Implementing milk processing will help to overcome low demand of milk during fasting period. This could be either by introducing a simple, low-cost agitator at

farm level or by using improved technologies at large scale. Farmers should be encouraged, trained and advised to pool their resources together in order to achieve this goal.

- Raising awareness for farmers by training on the presence of mastitis without symptom, its economic loss and means of preventing the disease through technical support from Jimma University aiming for good udder health for better quality milk production, income generation, and improving of living standard the farmers.
- Raising awareness to farmers contractual attachment with ambulatory clinic of the University will help them in improving herd health management, in getting artificial insemination and training and advice in control and prevention of mastitis and udder health for better quality milk production, income generation, and improving of their living standard.
- Training farmers to improve udder health
    - In general, at least the following practices "at herd level" are suggested to control mastitis in Jimma based in the results of this work:
        - ✓ Using a single towel for teat drying for each cow before milking reduces SCM in the herd.
        - ✓ Improving fertility on optimum moment
        - ✓ Allowing cows to be dried-off at a more appropriate moment reduces chronic infection.
        - ✓ Adding straw as bedding, improving housing conditions, teat dipping and disinfection reduces teat lesion,
        - ✓ Culling chronically infected cows, and replacing old cows,
        - ✓ Milking by squeezing instead of stripping reduces SCM in cows without udder tick infestation.

- ✓ Providing feed and water immediately after milking reduces quarter being blind.
- ✓ Controlling ticks by practicing zero-grazing and spraying acaricides whenever necessary.

6. Research gaps

6.1. Clinical mastitis incidence study

The prevalence of clinical mastitis in Ethiopia has been reported, also by us, but no studies on the incidence rate of clinical mastitis have been conducted before (Mungube et al., 2004; **Chapter 4.2**). Thus, further study is required to determine the incidence and average duration of clinical mastitis cases with the distribution of causative bacteria in Jimma. Such data would provide a basis for formulating a better mastitis prevention and control plan in the study area.

6.2. *Dry cow treatment study*

Dry cow treatment, as part of mastitis management, has not been practiced in Ethiopia. Yet, blanket dry cow treatment has been a cornerstone for mastitis prevention and control for many years (Boddie and Nicketson, 1986). Literatures indicated that clever use of antimicrobials at drying-off might prevent the need to treat cows during lactation. In an area like Jimma where emergence of antimicrobial resistance appears, the need for prudent use antimicrobials is without doubt mandatory. Hence, a clinical trial should be conducted to investigate if dry cow treatment is able to reduce the incidence of clinical mastitis in the early lactation period, and the overall prevalence of subclinical mastitis. Whether this will reduce

the usage of antimicrobial therapy at the time cows are lactating and the likelihood of finding antimicrobial residues in retailed milk are left to be investigated.

6.3. Molecular epidemiology of Staphylococcus aureus and *Staphylococcus* spp.

We found many people in Jimma are consuming raw milk. Also, the high prevalence of oxacillin-resistant *S. aureus* in raw bulk milk was investigated suggesting consumption of raw milk in Jimma might be a potential vehicle for the transmission of multidrug resistant staphylococci (**Chapter 3.1**). Molecular typing of strains from consumers, farmers, cows and bulk milk could test this hypothesis.

Staphylococcus spp. was the most frequently isolated pathogens in the quarters with clinical mastitis. Further studies are required to investigate whether *Staphylococcus* spp. isolates are more virulent in the Jimma strains compared with e.g. European and African isolates as well as whether susceptibility differs between dairy breeds (tropical and European) (Grootenhuis et al., 1979; Thorberg et al., 2009; Abrahmsén et al., 2014).

6.4. Nutritional study

Shortage of animal feed was one of the constraints identified (**Chapter 3**). Nutritional management such as silage/hay making and drying off cows during the dry period could partially solve the current feed shortage. A study comparing a regular feeding regime as a control group and a more ideal regime based on silage with balanced feeding from locally available feed resources as a treatment group could picture differences in milk yield, udder health, fertility, metabolic disease.

6.5. Ticks as a vector for mastitis pathogens?

Contagious mastitis pathogens can spread from cow to cow through flies (Bramley et al., 1985). A previous report in Zimbabwe indicates that ticks are a predisposing factor to udder and teat damage (Ndhlovu et al., 2009). Ticks can carry pathogens like the bacteria *Borrelia burgdorferi* (Wikel, 1999). The presence of many tick species have been reported in Ethiopia. *Amblyomma cohaerens, Amblyomma. variegatum, Boophilus. decoloratus, Hyalomma* spp. *and Rhipicephalus evertsi evertsi* are the dominant cattle tick species (Mekonnen et al., 2001). Higher odds of SCM in quarters from cows with tick-infested udder were reported in this finding, suggests that ticks might have a direct and/or indirect role in the disease occurrence. Whether the effect of ticks is due to direct damage or indirect as vectors for mastitis pathogens remains to be studied. On the other hand, ticks might undermine the cows immunity (Wikel, 1999) leading to higher mastitis susceptibility. Molecular typing of strains isolated from ticks, teats and milk and measuring the immune status of infested and non-infested cows could elucidate which explanation is most likely. The role of genetic in mastitis resistance and susceptibility needs to be studied as to select the best-fit optimal percentage of crossbreed blood level to the local conditions. Previous studies revealed that cattle with 50% exotic inheritance are best suited for dairying in Ethiopia (Haile et al., 2011).

7. Final conclusion

The findings can possibly used for awareness creation for consumers, producers, retailers and milk processing plants to improve milk quality and safety (**Chapter 3.1**). Also, policy makers can utilize the findings as an initial for dairy development policy preparation. Implementing of milk collection systems, establishing a milk quality lab and providing

training across the milk chain on hygienic farming practice and hygienic milk handling are problems that should be solved without wasting time to add value for quality milk supply in Jimma. In **chapter 4.1 and 4.2,** manageable risk factors associated with presence or absence of subclinical mastitis, clinical mastitis, and IMI cases were identified. In these studies, Jimma specific possible mastitis prevention reported are drying of teats before milking, milking by squeezing instead of stripping, and application of appropriate tick control measurements and creating awareness and training farmers to apply these results in farm management could help to improve the milk quality and udder health. Above all, these findings could help for designing prevention and control strategy of mastitis for the local Ethiopian condition and neighboring countries.

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SUMMARY – SAMENVATTING - GOOLABA

SUMMARY

Mastitis remains an economically important disease in the dairy industry worldwide. In **Chapter 1**, a review on diagnosis, etiology, prevalence, risk factors and control of mastitis are given and the advantages and disadvantages of control measures available are discussed. Dairy consumption as well as production systems differ among regions within Ethiopia.

The general aims of this thesis were to investigate milk production, quality and consumption and study the prevalence of mastitis and associated risk factors at herd-, cow- and quarter-level in Jimma, Ethiopia (**Chapter 2**).

Milk production, marketing, quality, consumption and its associated constraints in Jimma were pictured through four studies **(Chapter 3.1)**. First, 47 dairy farmers and 44 milk retailers were interviewed to gain more insights in dairy farming and marketing, and associated constraints. Second, bulk milk samples (n = 188) were collected for 4 consecutive weeks to investigate milk quality [Total Bacterial Counts (TBC), Coliform Counts (CC), Somatic Cell Counts (SCC), and antimicrobial residues]. Third, (bulk) milk samples from 32 farms, 46 milk retailers and the 3 local milk collection centers were collected to determine the presence of oxacillin susceptible–and oxacillin resistant *Staphylococcus aureus* isolates. Fourth, 208 adult inhabitants were interviewed to gain more insight in milk consumption and concerns of consumers. The average dairy farm included in the studies consisted of 5 lactating cows, produced 43 liters of milk per day and was owned by male, literate adults. Milk was sold to retailers (71% of the production) and directly to customers (25%) without any quality control, whereas 4% was self-consumed. Shortage of animal nutrition and adulteration of the milk were the main constraints for farmers and retailers, respectively. The median TBC, CC and SCC were 122,500 CFU/mL, 1005 CFU/mL and 609,500 cells/mL, respectively. Antimicrobial residues were detected in 20% of all milk samples. In general, the milk quality

was considered to be poor (TBC> 10,000 CFU/mL, and/or CC > 100 CFU/mL, and/or SCC> 400,000 cells/mL and/or presence of antimicrobial residues) in 97% of all samples. *Staphylococcus aureus* was isolated from 12 (38%), 13 (33%), and 2 out of 3 of the milk samples originating from the dairy farms, the milk retailers, and the milk collection centers, respectively. Seven (26%) of the isolates were resistant to oxacillin suggesting the presence of Methicillin Resistant *Staphylococcus aureus* or MRSA (Lee, 2003). Locally produced milk is occasionally consumed by adults but more frequently by children. Adults mainly drink spontaneously fermented milk whereas most milk for children is boiled. Most consumers are concerned about adulteration and milk borne diseases but not about the presence of antimicrobial residues. Educated consumers (secondary school or higher) were more likely to boil milk for own consumption, to be concerned about the presence of antimicrobial residues in milk, to be concerned about milk-borne diseases and willing to pay more for milk with proven good quality compared to poorly educated consumers.

In **Chapter 4.1**, the prevalence of subclinical mastitis and associated risk factors at the herd, cow and quarter level were studied in smallholder dairy farms in Jimma, Ethiopia. Forty-two herds were visited, a questionnaire was performed, and 635 quarters belonging to 176 lactating cows were screened to detect the presence of subclinical mastitis using the California Mastitis Test. Sixty-two % of the cows and 51% of the quarters had subclinical mastitis. Quarters from cows in later stage of lactation (>180 days in milk) [opposed to early lactation (<90 days in milk)] and quarters from cows with a tick-infested udder had higher odds of subclinical mastitis, as reflected by the California Mastitis Test. Also, quarters from cows without udder tick infestations milked by squeezing were less likely to suffer from subclinical mastitis than when milked by stripping. However, the milking technique did not influence the odds of mastitis in cows with ticks on the udder. This study stresses the high

prevalence of subclinical mastitis in smallholder dairy farms in Jimma and a lack of awareness of the existence of the disease among dairy farmers.

In **Chapter 4.2**, a cross-sectional study on clinical mastitis, intramammary infection and blind quarters was conducted. Risk factors at the herd, cow, and quarter level for clinical mastitis and (pathogen-specific) intramammary infection were studied using multilevel modeling and factors associated with quarters being blind were also investigated. Eleven percent of the cows and 4% of the quarters had clinical mastitis whereas 85% of the cows and 51% of the quarters were infected. Most of the cows had one or more blind quarter(s), whereas 6% of the quarters were blind. Non-*aureus staphylococci* were the most frequently isolated pathogens from the clinical mastitis cases and causing most of the intramammary infections. The odds of clinical mastitis was lower in herds where heifers were purchased in the last year, old cows (>4 y) and quarters not showing teat injury. The odds of intramammary infection caused by any pathogen was higher in herds not practicing teat drying before milking (opposed to drying teats with 1 towel per cow), late lactation cows (> 180 days in milk opposed to ≤90 days in milk), cows with high body condition score (>3)], right quarters (opposed to left quarter position) and quarters showing teat injury. Quarters of cows in herds with bucket-fed calf feeding (opposed to suckling) had higher odds of intramammary infection caused by *Staphylococcus aureus*. Except for body condition score, intramammary infection caused by non-*aureus staphylococci* was associated with the same risk factors as intramammary infection caused by any pathogen. No access to feed and water immediately after milking, higher parity and tick infestation were risk factors for quarters being blind.

In **Chapter 5,** results from the preceding chapters are discussed with a focus on the practical applications and research gaps.

SAMENVATTING

Mastitis blijft wereldwijd een economisch belangrijke ziekte voor de zuivelindustrie. In **Hoofdstuk 1** wordt ingegaan op de diagnose, etiologie, het voorkomen, geassocieerde risicofactoren en de controle van mastitis en worden de voor- en nadelen van beschikbare controlemaatregelen bediscussieerd. Dat de zuivelconsumptie en de productiesystemen verschillen tussen de regio's in Ethiopië, wordt vastgesteld.

De algemene doelstellingen van deze thesis omvatten het bestuderen van de melkproductie, de melkkwaliteit, en de melkconsumptie in Jimma, Ethiopië, naast het voorkomen van mastitis en de ermee geassocieerde risicofactoren op bedrijfs-, koe-, en kwartierniveau (**Hoofdstuk 2**).

De melkproductie, de marketing, de melkkwaliteit en de melkconsumptie in Jimma werden in kaart gebracht samen met de ermee geassocieerde beperkingen op basis van 4 studies (**Hoofdstuk 3.1**). Eerst werden 47 melkveehouders en 44 melkhandelaren geïnterviewd om meer inzicht te verwerven in de melkveehouderij en marketing en de ermee geassocieerde uitdagingen. Ten tweede werden gedurende 4 opeenvolgende weken tankmelkstalen (n = 188) verzameld om de melkkwaliteit (totaal kiemgetal, coligetal, celgetal en antimicrobiële residuen) te bestuderen. Ten derde werden (tank-) melkstalen op 32 melkveebedrijven, en bij 46 melkverkopers en 3 lokale melkcollectiecentra verzameld om de aanwezigheid van oxacilline-gevoelige en oxacilline-resistente*Staphylococcus aureus* isolaten na te gaan. Ten vierde werden 208 volwassen inwoners geïnterviewd om meer inzicht te verwerven in u melkconsumptie en ermee geassocieerde bezorgdheden. Het gemiddeld melkveebedrijf uit de studie had 5 stuks lacterend melkvee, produceerde 43 liter melk per dag en was de eigendom van mannelijke, geletterde volwassenen. Melk werd verkocht aan kleinhandelaren (71% van de productie) en direct aan consumenten (25%) zonder enige kwaliteitscontrole, terwijl 4%

zelf werd geconsumeerd. Respectievelijk een tekort aan voeding voor de dieren en fraude met de melk werden gezien als de belangrijkste beperkingen door veehouders en verkopers. Het mediaan van het kiemgetal, coligetal, en celgetal bedroeg respectievelijk 122,500 kolonievormende eenheden, 1005 kolonievormende eenheden en 609,500 cellen per milliliter melk. Antimicrobiële residuen werden gedetecteerd in 20% van alle melkstalen. Over het algemeen werd de melkkwaliteit in 97% van de melkstalen als inferieur (totaal kiemgetal > 10,000 kolonievormende eenheden, en/of coligetal > 100 kolonievormende eenheden, en/of celgetal > 400,000 cellen per milliliter melk, en/of de aanwezigheid van antimicrobiële residuen) bestempeld.*Staphylococcus aureus* werd geïsoleerd uit respectievelijk 12 (38%), 13 (33%) en 2 van de 3 melkstalen afkomstig van de melkveebedrijven, de melkhandelaren en de melkcollectiecentra.Zeven (26%) van de isolaten waren oxacilline-resistent wat wijst in de richting van methicillineresistente *Staphylococcus aureus* of MRSA. Lokaal geproduceerde melk wordt af en toe door volwassen maar vooral door kinderen geconsumeerd. Volwassenen drinken vooral spontaan-gefermenteerde melk terwijl de meeste melk geconsumeerd door kinderen gekookt wordt. De meeste consumenten maken zich zorgen over fraude met melk en ziektes verspreid door melk maar niet over de aanwezigheid van antibioticaresiduen. Opgeleide consumenten (middelbare school of hoger) kookten vaker hun melk voor eigen consumptie, waren bezorgder over de aanwezigheid van antimicrobiële residuen in melk en over ziektes die via melk worden overgedragen en zijn bereid meer te betalen voor melk van bewezen goede kwaliteit in vergelijking met minder opgeleide consumenten.

In **Hoofdstuk 4.1**, werden de prevalentie van subklinische mastitis en geassocieerde risicofactoren op bedrijfs-, koe- en kwartierniveau bestudeerd op kleinschalige melkveebedrijven in Jimma, Ethiopië. Tweeënveertig melkveebedrijven werden bezocht, een enquête werd afgenomen, en 635 kwartieren van 176 lacterende melkkoeien werden gescreend op de aanwezigheid van subklinische mastitis met de California Mastitis Test.

Tweeënzestig % van de koeien en 51% van de kwartieren hadden subklinische mastitis. Kwartieren van koeien in een later lactatiestadium (>180 dagen in lactatie) [versus vroege lactatie (<90 dagen in lactatie)] en kwartieren van koeien met uiers geïnfesteerd door teken hadden vaker subklinische mastitis, zoals gereflecteerd door de California Mastitis Test. Ook hadden kwartieren van koeien zonder teekinfestatie en gemolken door masseren minder vaak subklinische mastitis dan wanneer gemolken door strippen. Evenwel had de melktechniek geen invloed op de aanwezigheid van mastitis bij koeien met teken op de uier. Deze studie benadrukt de hoge subklinische mastitis prevalentie in kleinschalige melkveebedrijven in Jimma en het gebrek aan besef rond het bestaan van deze ziekte onder melkveehouders.

In **Hoofdstuk 4.2**, werd een crosssectionele studie gedaan naar klinische mastitis, intramammaire infectie en blinde kwartieren.Risicofactoren op bedrijfs-, koe- en kwartierniveau voor klinische mastitis en pathogeen-specifieke intramammaire infectie werden bestudeerd door "multilevelmodelling" terwijl ook factoren geassocieerd met niet-producerende kwartieren werden onderzocht. Elf % van de koeien en 4% van de kwartieren hadden klinische mastitis terwijl 85% van de koeien en 51% van de kwartieren geïnfecteerd waren. De meeste koeien hadden 1 of meer niet-producerende kwartieren en in totaal produceerde 6% van de kwartieren niet. Niet-*aureus stafylokokken* waren de vaakst geïsoleerde pathogenen bij de klinische mastitis gevallen en veroorzaakten de meeste intramammaire infecties. De kans op klinische mastitis was lager op bedrijven waar in het laatste jaar vaarzen werden aangekocht, bij oudere koeien (>4 jaar) en in kwartieren zonder speenletsel. De kans op intramammaire infectie veroorzaakt door om het even welke pathogeen was hoger op bedrijven waar de spenen niet werden afgedroogd voor melken (versus afdrogen van de spenen met 1 doek per koe), bij koeien in late lactatie (>180 dagen in lactatie versus ≤ 90 dagen in lactatie), bij koeien met een hoge lichaamsconditiescore (>3), in rechterkwartieren (versus linkerkwartieren) and in kwartieren met een speenletsel. Kwartieren

van koeien op bedrijven waar kalveren gevoed worden met de emmer (versus zuigen bij de koe) maakten dan weer meer kans om geïnfecteerd te zijn met *Staphylococcus aureus*. Met de uitzondering van lichaamsconditiescore, waren de risicofactoren geassocieerd intramammaire infectie veroorzaakt door niet-*aureus stafylokokken* dezelfde als die voor intramammaire infectie veroorzaakt door om het even welke pathogeen. De kans op niet-producerende kwartieren was groter op bedrijven waar de koeien geen toegang hadden tot voeder en water direct na melken, bij koeien met een hogere pariteit en met een tekeninfestatie.

In **Hoofdstuk 5** worden de resultaten van de vorige hoofdstukken besproken met een focus op de praktische toepassingen en de lacunes in het onderzoek.

GOOLABA

Dhukubni gur'aa (maastayitiin) dhukuba hubaatii dinagde geesisun industirii annannii kessattii duuadugnaa marairratti beekamuudha. **Boqonnaa 1** keessatti**,** sakatta'insa ka'umsa, faca'insaa fi danqaa fi to'annoo dhukkubii gur'aa ibsuun, balaa fi bu'aa mala to'annoo dhukkuba gur'aa gadi fageenyaan mari'atameera. Itoophiyaa keessatti haallii soorratama aannanii fi hoomishaamni naannoo irraa naannootti garaagaruummaa ni qaba.

Dimshaashumatti, kaayyoon baruu kanaa hoomishaa, qulqullinaa fi akkataaa soorratama aannnanii qorachuudha. Akkasumas faca'insaa dhukkuba gur'aa fi isaa waliin walqabatuu rakkoo adarkaa hoomaa, sawaa fi muchaatti Jimmaa Itoophiyaa keessatti mul'atu xiinxaluudha (**Boqonnaa 2**).

Hoomiisha aannanii, gabaa, qulqullinaa fi sorrata akkasumas rakkoo kanaan walqabate irratti qoraannoon geggeefameera (**Boqonnaa 3.1**). Jalqaba irratti qonnaan bultoonnii 47 fi namootii aannan gurguran 44 rakkoolee aananni hoomishuufi gurguruu akkasumas rakkoolee isaa waliin walqabatan irrati kan afgaafiin kan dhiiyaateefiidha. Lammata irratti iddatoon [n=188) aannanii turban afuriifii walitti aanusuun fudhachuun qulqullinni aannanii qoratameera. Hundi baakeeriyaa lakka'aman (TBC) lakkoofsii kooliifoormii(CC) seelliin qaamaa lakka'ame (SCC) harcaatuu farra maayikrooyootaf]. Sadaffaa iddottoon aannaan baayyee qonnaan bultoota 32, aannana gurgurtoota 46 fi giddu gala aannan kanwalitti qaban naanna'atti argaman sadii irraa walitti guuramee akka isaan oksaasiliin of keessaa qabanii fi oksaasiliin kan ofirraa ittisanii yookaan damdamatan *Staafeelookookas awuras* murteessuuf yaalameera. Afraffaa ,ga'eessota 208 naannaa kana jiraatan irraa oduu qorachuun haala soorata aannanii fi isaa waliin kan walqabatee haala sooratoota aannanii irratti gadifageenyaan hubannoo argachuuf yaalameera. Giddugaleessaan horsiisii aannan loonii qorannoo kana keessatti hammataman saawwan shan ilmaman guyyaatti liitiraa 43 kan keennanii yoo ta'u

abbaan qabeenya kanas irra jiran isaanii dhiiraa fi nama barataniidha. Aannaanii hoomeeshamee keessa (%71) namoota qanxaboo gurguranitti raabsama, harkii (%25) ammoo kallattanii osoo qulqullinnii isaa tokkoyyuu hinlaalamiin namootii sorratan fudhatu; akkasumas kan hafee harkii (%4) immoo namootoo hoomishaniin fayadama irra ola. Hirri'inni nyaata yookaan soorrata loon aannanii fi aannanitti bishan dabaluu rakkolee ciccimoo qonnaan bulaa fi warra qanxaboo gurguraan walduraa dubaan mudatuudha. Midiyaan(waaltosaa) TBC, CC fi SCC isaa 122,500 CFU/mL, 1,005CFU/mL fi 609,500 cell/mL walduraa dubaan ta'un isaa beekameera. Sadarkaa %20tti harcaatuun farra maayikroobaayalii iddatoo aannaii qorataman hunda keessatti mul'ateera. Dimshaashumaatti qulqulliinnii aannaniichaa yaraa ta'un [(TBC>10,000CFU/mL fi yookaan CC>100CFU/mL akkasumas, SCC>400,000Cell/mL fi yookaan harcaatuu farraa maayikroobaayaaliis aragame] %97 idddatoo aannaii hundaa keessatti argameera. Iddattoo aannanii horiisaa qonnaa aannan loonii rraa 12(%33), warraa aannan qanxaboo gurguran irra 13 (%38) fi 2 bakka sadii irraa waltiitti qabamee keessatti *Staafeelookookas awuras* argamuun isaa mirka'aneera. Adaan foyaametee torba keessaa (%26) oksaasiliin kan damdamatuu ta'uun isaa jireenya MRSA mirkaneessa (Lii, 2003). Aannani naann'atti hoomishaaman darbee darbee kan dhugaman ga'eessota yoo ta'u yeroo baayyee garuu aannan kan dhugani daa'immani. Ga'eessotti irraa guddeessi akka tasaa aannan ititee darbee darbee kan dhugan yoo ta'u; yeroo mara garuu daa'imman aannan danfsamee dhugama. Irra caalaan soorratoota aannanii kan dubbatan waa'ee waan biran ananni walmaku fi dhukkuba aannanii irra namatti dhufaan malee waa'ee harcaatuu farra maayirkoobalii miti. Namootii barnoota qaban fakkeenyaaf barumsaa sadarkaa lammaf fii isa ol qaban aannan danfiisaanii kan soorratan yoo ta'u rakkoo harcaaftuu farraa maayikiroobayalii, rakkoo dhukkuba aannan irraa nama qabuu irratti baayyee dhiphatu; akkasumas annani qulqullinaa hinqabnee irraa aannan qulqullinaa isaa mirkaana'ee gatii olaanan bituuf feedhii guddaa qabu.

Boqonnaa 4.1 keessatti, faca'insi dhukuba gur'aa kan ijatti hin mul'anne (subclinical mastitis) fi rakkooleen isaan walqabatan sadarkaa hoomaatti, sawaa fi muchaa qonnan bultoota qabeenyaa horiisa loonii xiqqaa qaban magalia Jimma, Itoophiyaa keessatti argaman irraatti qoratameera. Hoomaa 42tu ilaalame, afgaafiinis ni godhame akkasumas muchi 635 sawwan elmaman 176 dhukuba gur'aa kan ijatti hin mul'anne qabaachuu isaniif kaalifoorniyaa qora dhukkuba gur'aa fayadamuun adaba;eera. Sawwan %62 fi mucha %51 dhukuba gur'aa kan ijatti hin mul'anne qabu. Muchi saawwan boodaa yookaan orogaa hosiisan (guyya 180 oli) fallaa dura hosiisan (guyya 90 gadii) fi muchi saawwa gur'aa irra silmii qabuu dhukuba gur'aa kan ijatti hin mul'anneen olaanatti kan qaban ta'u kaalifoorniyaa qora dhukkuba gur'aa godhameratti mula'teera. Akkasumas, muchi saawwa gur'ii silimi hin qabne, kan quba shananii elmamu isa quba sadiin elmamu gadi carra dhukkuba gur'aa kan ijatti hin mul'annen kanaan qabamu qaba. Haa ta'u malee, haalli elmaa kun gur'aa silmii hin qabne irratti dhiibaa hin qabu. Qorannon kun faca'insi dhukuba gur'aa horsiis loon ananni Jimma ol ka'aa ta'uu fi warreen loon horsiisan dhukubni kun jiraachuu kan quba hin qabne ta'uu isaa argisiisa.

Boqonnaa 4.2 keessatti, qorannoon kutalee qaxxaamuruu dhukuba gu'raa ijatti mulatu (clinical mastitis), infeekshiin gu'raa kessaa (intramammary infection), fi much duudaa irraatti geggeefameera. Rakkoon balaaf hoomaa, saawa fi sadarkaa mucha isaan qunnamuu dhukuba gu'raa ijatti mulatu fi dhukkuboot infeekshinii gur'aa keessaa (IMI) moodala sadarkaa heedduu godhachuun geeggeefame, akkasumas mucha duudaa irraatti godhameera. Saawwan dhibbeentaa (%11) fi mucha (%4) dhukuba gu'raa ijatti mulatu qabu akkasumas saawwan dibbeentaa (%85) fi muchi %51 infeekshiin gu'raa kessaa (intramammary infection) kanaan qabamaniiru. Saawwan irra caalan muchaa tokkoo yookaan muchaa ol duuda qabu, akkasumas mucha dibbantaa (%6) duuda dha. Hinta'iin-*Staafeelookookas awuraas* dhukuba gu'raa ijatti mulatu (clinical mastitis), fi infeekshiin gu'raa kessaa (intramammary infection) yeroo mara mul'attanii adda baafamaniidha. Carraan dhukuba gu'raa ijatti mulatu hoomaa

raddeen baayeen bara darban bitaman fi saawwan dolloomoo (waggaa 4 olii) akkasumas mucha fiixeen isaa madaa hinqabnerattii dha. Akkasumas, carraa infeekshiin gu'raa kessaa paatoojiinii kamiinuu dhufuuf qabu hoomaa fiixeeen muchaa saawwa fooxaa tokkoon gogosaman irra hoomaa tasuma fiixee muchaa qorsuun hingeggeefameene keessatti irra caala. Saawwan boodaa yookaan orogaa hosiisan guyyaa180 ol (fallaa guyyaa 90 gadi), sawwan haalii qaama isaan olaanoo ta'an (BSC)>(3)], mucha afran keessan kan bitaa fi kan mirga irraatti argamu) akkasumas fiixee mucha madoo irratti dha. Muchii saawwanii hoomoo keessatti jabiileen baaldiin sooramaniifi warra hodhan irraa infeekshiin gu'raa kessaa *Staafeelookookas awuraasiin* carraa isaan dhufuu qaban guuddaadha. Haalii qaama isaan olaanoo ta'an (BSc) irraa kan hafee IMIiin *Staafeelookookas awurasii* hintaaneen kana dhufaan rakkoolee walfakkatoo paatoojiinii kamiin dhufan waliin kan IMI dhufan tokkoo dha. Erga elmaamanii boodaa nyaataa fi bishaan yeroosuma argahuu dhabuun, saawan baay'e dhalan fi silmiin rakkoolee guddoo muchaa saawwa doomisanii dha.

Boqonnaa 5, keessatti bu'aan qorannoo boqonnaalee dursan keessatti itti fayyadama qabatamaa irraatti bu'ureeffameen irraatti mari'atamee fi hir'inni qorannoos agarsiifameera.

CURRICULUM VITAE

Curriculum vitae

Tadele Tolosa was born in Bantu, central Oromia, Ethiopa. Attended his elementry and junior seconday school at Bentu elementry and junior seconday school. He has also followed his senior seconday school at Sebeta, 25 km west of Finfine (Addis Ababa), the capital of Oromia and Ethiopia.

In 1983, he joined Addis Ababa University (AAU), Faculty of Veterinary Medicine at Debre zeit. He specialiizes in the field of veterinary medicine. After graduation from AAU, he worked in different localities of the country as a provincial veterinarian, project coordinator and senior veterinary officer of Jimma Zone Agricultural Office, Oromia state. Then, in October 2002 he joined Addis Ababa University, Faculty of Veterinary Medicine to pursue his Masters in Tropical Veterinary Medicine. In september 2004, he joined Jimma University as assistant professor of microbiology.

In September 2010, he got a chance to visit the Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University through JU-IUC programme. In September 2011 he started his PhD at the Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University through JU-IUC programme. His PhD research was entitled: *Milk Quality and Mastitis in Jimma, Ethiopia-Risk Factors and Constraints*. Tadele has established an ambulatory clinic and mastitis and milk quality laboratory at Jimma University, Ethiopia. He is the head of mastitis and milk quality laboratory. Tadele is married and a father of two sons.

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